THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND ~~SOIL~~ GROWTH

plant

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INTRODUCTION

The use of heat-sterilized soils in methods of research in soil biology and plant pathology as well as in various phases of practical agriculture is rapidly increasing in value. For purposes of research especially, the sterilization of soil by heat is practically the only method which can be relied upon for purposes of rendering the soil sterile as regards any particular organism. Since the application of heat to the soil may, and usually does, result in important changes aside from sterilization which may greatly influence plant growth, it is evident that a knowledge of the alterations which occur or may be expected to occur in the soil is essential in order to draw reliable conclusions from certain types of research conducted with heated soils. The problem is by no means a new one. A great variety of literature exists upon the subject of soil sterilization from various angles of attack. Since the subject may be treated from the standpoint of the soil itself as chemistry, biology, or physics, or from the standpoint of plants grown in these soils as physiology or pathology, the field has been a very productive one. At the same time, its complexity has led to quite widely varying results and conclusions. Although it is evident that investigations in this field should take into account all factors which may be involved before drawing far-reaching conclusions, it is also evident that it is exceedingly difficult to grasp the significance of, and lay proportionate weight upon all the factors concerned. The results presented in this paper were first undertaken from a phytopathological point of view. The bearing of the conclusions drawn on phytopathological research are contemplated in another paper. It is the purpose of this paper to present the data obtained on the changes produced in heated soils as measured by their influence on seed germination and plant growth, and to discuss the probable nature of these changes.

A clear conception of the subject of soil sterilization requires at the outset a good understanding of the methods by which soils may be sterilized. These are: (a) Sterilization by heat, and (b) sterilization by chemicals. The former may again be divided into methods using (a) live steam, and (b) dry heat; the latter into methods using (a) volatile antiseptics, and (b) non-volatile antiseptics. Clearly these methods may be expected to yield funda-

mentally different results, not only in regard to the method employed, but also in regard to the intensity with which it is applied. Technically the term sterilization means the complete destruction of all living matter in a medium, and implies the continued sterility of this medium if desired. This term as used in connection with the treatment of the soil, however, does not necessarily mean the complete destruction of all living matter in the soil nor its continued sterility. Ordinarily it refers to the destruction of one or more specific organisms which are not desired in the soil with no special precautions to prevent reinfestation. In a broader sense, however, the object of soil sterilization is the production of a more favorable medium for the growth of cultivated plants by means of heat or chemical disinfection. This may be brought about as much by the improvement of the chemical, physical, or biological relationships in the soil, as by the simple destruction of one or more forms of undesirable organisms. In view of these facts the terms "partial sterilization" and "pasteurization" have come to be used by some investigators.

The object of soil sterilization in practical agriculture is, therefore, usually either the destruction of some particular plant parasite harbored in the soil, or the improvement of the fertility of the soil by affecting its chemical, biological or physical condition. Where it is practiced for the former reason, the latter results are naturally also secured as secondary effects. Many farmers are, for instance, coming to regard the process as valuable from the standpoint of destruction of weed seeds in plant beds alone.

Various methods of application have been devised with the result that the practice has been increasing rapidly in certain forms of intensive plant culture. Up to the present time, soil sterilization has been found most useful and has received its greatest impetus in the culture of plants under glass. The vegetable forcing house industry particularly, has used the method on a large scale. Florists use it less commonly, but find it advantageous for certain plants and for starting seed. In the culture of tobacco, ginseng, coniferous seedlings, and most seedlings for the market gardener, it has been found well worthy of use.

BRIEF SUMMARY OF LITERATURE

The beneficial action of heated soils upon plant growth was observed long before the sterilizing value of heat was known. According to Sir Humphrey Davy (12) the improvement of "sterile" lands by burning was known to the ancient Romans, the custom having been mentioned by Virgil in his first book of the Georgics. It is well known that the burning of various types of soil became quite general in Europe during the eighteenth century. This method was accompanied by a considerable amount of scientific investigation upon the subject, until the practice fell into disuse about the middle of the last century.

The actual use of sterilizing agents upon the soil for the primary purpose of destroying injurious forms of living organisms present in the soil is largely a

development of the past three or four decades. Accompanying these studies and practices of soil sterilization for the destruction of soil organisms, there has accumulated a great deal of literature dealing with the scientific principles involved. These publications have been especially stimulated by a desire to explain the reason for the increased growth of plants on sterilized soils. The investigations undertaken from quite different angles of attack and with great variation in type of soil, kind and intensity of sterilizing agents, as well as in type of plants used, have quite naturally resulted in widely varying conclusions.

A detailed review of the literature concerning soil sterilization would be too voluminous to present here. For present purposes it may suffice to present in brief form an outline of the principal changes produced in and by sterilized soils, and to mention briefly certain theories concerning the nature of the action of sterilized soils on plant growth, leaving the discussion of the literature more directly concerned with this investigation to be treated under the separate phases of the subject as they are taken up.

Principal changes produced in and by sterilized soils.

I. Destruction of life.
   - A. *Normal soil flora and fauna,* desirable and undesirable forms of bacteria, fungi, protozoa, and higher animals.
   - B. *Plant parasites,* especially pathogenic bacteria, fungi, nematodes, and injurious soil infesting insects.
   - C. *Propagative organs of higher plants,* especially weed seeds.

II. Immediate chemical action (formation of toxic and beneficial compounds).
   - A. *Decomposition of organic material* resulting in formation of ammonia, carbon dioxide and various new and complex organic compounds.
   - B. *Decomposition of inorganic material,* reduction of nitrates and nitrites to ammonia and increased solubility of potassium, phosphorus and other salts.

III. Bio-chemical action.
   - A. *Increased ammonification* particularly and modified nitrification, denitrification, and nitrogen fixation.

IV. Physical action.
   - A. *Absorptive capacity of soil* modified for water, gases, and salts.
   - B. *Increased concentration of soil solution.*
   - C. Modified capillarity, colloidal state, and mechanical condition.
   - D. Modified color and odor.

V. Action on organisms growing in sterilized soils.
   - A. Lower organisms.
      1. Increased development due to reduced competition, increased food supply, destruction of "bacterio-toxins," "stimulation" by products added or formed, or other causes.
      2. Retardation in growth in rare cases due to injurious conditions produced.
   - B. Green plants.
      1. Injurious action as indicated by retarded rate and percentage of seed germination and by retarded rate of plant growth.
      2. Beneficial action as shown by increased rate and percentage of seed germination and increased rate and amount of plant growth.
      3. Modification in form, color, and other "qualitative" changes.

The theories which have been promulgated by various investigators rest primarily upon the explanation of the beneficial and injurious action of sterilized soils upon plant growth. These theories are partially interrelated and do not permit of satisfactory classification, but the main ones may be grouped as follows:

1. Stimulation hypothesis. Koch's (31) theory formulated in 1899 was probably the first attempt at scientific explanation of observed facts. He believed the sterilizing agent or its products directly "stimulated" the growth of the plants or soil bacteria, which in turn influenced plant growth.

2. Modified bacterial activity. Hiltner and Störmer (25) in 1903 showed by soil bacterial counts that sterilization resulted in an initial decrease in numbers of organisms followed by a marked increase in numbers, and hence an increase in the productiveness of the soil.

3. Protozoan theory. Russell and Hutchinson (62) believe that the benefit from "partial sterilization" may result from the destruction of the larger phagocytic microörganisms (mostly protozoans) which inhibit the development of the beneficial organisms. The protozoa being destroyed, the ammonifying bacteria, for instance, increase rapidly producing an increase in nitrogen and hence plant growth.

4. "Bacterio-toxin" theory. Grieg-Smith (22) considers that certain substances which he calls "bacterio-toxins" and which exist naturally in soils, inhibiting bacterial growth, are destroyed by sterilizing agents.

5. Modified organic soil compounds. Schreiner and Lathrop (65) believed that certain complex organic soil constituents produced as a result of sterilization by heat, may increase plant growth while others inhibit it. That organic soil constituents of an indefinite nature were produced which were injurious to plant growth in heated soils had also been suggested by Struckman (73), Dietrich (13) and Pickering (52).

6. Modified inorganic soil compounds. This theory though supported by no investigators in particular should be added here since it has been repeatedly shown since the time of Struckman (73) that increase in inorganic plant food constituents occurs in heated soils. The theory is perhaps best supported by Liebscher (40) who believed that sterilization was essentially nitrogenous fertilization.

7. Plant parasite theories. The fact that in certain soils the benefit of soil sterilization may be due largely to the destruction of parasitic organisms is unquestionable. The wide application of this theory, however, to the subject in question, as supported perhaps most energetically by Bolley (5), serves to place this type of response to sterilization among the theories explaining the action of sterilized soils.

8. Physical theories. These theories are not subscribed to by any author in particular at the present time, although it was quite generally believed at one time that all the benefit derived from burning the soil was due to purely physical changes. Some of the physical factors which play a part in soil fer-

tility are, however, coming to be regarded as very influential in conjunction with chemical factors. Seaver and Clark's (67) "concentration theory" may, for instance, properly be placed here.

For a more detailed summary of the subject of soil sterilization especially as regards the relation of protozoa to sterilized soils, the recent review of Kopeloff and Coleman (34) should be consulted.

EXPERIMENTAL WORK

The investigations upon the subject of soil sterilization were begun by the writer in 1909 with the object of studying methods for the control of the damping-off disease of plants, caused by *Pythium debaryanum*, and *Rhizoctonia*. At the outset, it became evident that when soils were treated either with heat or chemicals, various "secondary effects" of sterilization occurred, which modified the soil and plant growth aside from the control of disease. Following a publication upon the control of damping-off in plant beds (27), the study of these "secondary effects" was undertaken. The primary object of this study was to attempt to find an explanation for the injurious action followed by the beneficial action of sterilized soils on plant growth. On account of the size and complexity of the problem, it became necessary to limit the investigation primarily to the effects of sterilization by heat. The studies up to date have been mainly concerned with the action of heated soils upon seed germination and plant growth; although various other phases have been taken up from time to time with the general idea of rounding out the problem in such a way as to make it of value also in the plant pathological investigations carried on simultaneously. In carrying on this work the soil has in many cases been heated to a degree far above that which is used in ordinary sterilization. While no apology may be necessary for such procedure from a chemical standpoint, the general idea has been to increase the action so that there would be produced a sufficient magnification of effect to obtain a good theoretical working basis for the explanation of similar results secured at ordinary temperatures of sterilization. While comparisons cannot readily be made with the "partial sterilization" of Russell and his associates, (62, 64), yet it is hoped that some evidence of value in this connection may be gathered from the results. The work presented here is perhaps more comparable to that of Pickering (50–53), or Seaver and Clark (67, 68), than to that of other investigators. On the other hand the writer has gone into the subject with a desire to bring together the various phases of the subject as presented in the literature with the idea of rounding out the problem and attempting to clear up some of the obscure points.

Materials and methods

The different soils used were selected largely for their variations in general type, especially in physical structure, rather than for differences in their chemi-

cal properties or their productivity. Their names will, therefore, quite satisfactorily indicate the general nature of these soils. No complete mechanical analyses have been made, but a determination of the loss on ignition indicates the range of organic matter present in the various soils as well as the general character of each soil in regard to this constituent. The determinations of total nitrogen, phosphorus, and potassium serve to give an idea of the state of fertility of these soils as far as these elements are concerned. The general character of the soils used is summarized in table 1.[1]

TABLE 1

General character of soils used in the experimental work

NAME	LOSS ON IGNITION	N	P_2O_5	K_2O	SOURCE	GENERAL DESCRIPTION
	per cent	*per cent*	*per cent*	*per cent*		
Waukesha silt loam	5.4	0.26	0.056	1.65	Milton, Wisconsin	Dark, tillable, fairly productive
Muck	14.8	0.65	0.14	1.52	Station farm, Madison	From drained cropped land, fairly fertile but responding to nitrogen
Virgin sandy loam	5.6	0.26	0.054	0.66	Station farm, Madison	Dark, good texture, not fertile from wooded pasture land
Miami silt loam		0.20	0.07	1.69	Station farm, Madison	From field cropped 10 years to tobacco but heavily fertilized
Fine sandy loam	4.3	0.14	0.068	1.20	Station orchard, Madison	A medium light soil containing considerable silt, not very productive
Peat	68.1	2.82			Station farm, Madison	A thoroughly decomposed peat, previously cropped and quite fertile
Norfolk sand	1.9	0.05			Upper Marlboro, Maryland	A very light sand, relatively low in productivity
Red clay	5.5	0.02			Ashland, Wisconsin	Very heavy red clay and non-productive

The soils were taken from the fields during the fall and allowed to air dry for most of the work where dry heat was used. The methods of heating the soils were usually of two kinds: First by steam in the autoclave at which a temperature of 114°-116°C. was maintained for one and one-half hour, or second by dry heat, in gas, electric, or hot water ovens. For temperatures of 500°C. or

[1] The writer is indebted to the Soils Department, University of Wisconsin, for the determinations of nitrogen, potassium, and phosphorus.

over the electric furnace was used. The time of heating was usually brought as near to two hours as possible.

When the work was first started and in fact during the greater part of this investigation, it was supposed that the time of heating the soil was of comparatively small consequence. The soil was usually, therefore, kept at the desired temperature for only a comparatively short period of time, the heat being allowed to increase gradually in the dry ovens for the first hour. Later on it became evident that a source of experimental error lay in the length of time of heating and the lack of uniformity of heat distribution in large samples of soil. Where large quantities of soil were heated, they were usually spread out in the ovens in a layer about one inch thick. This was also true when heating was done in the autoclave for short periods of time. The temperatures were taken either by means of a mercury or electrical thermometer for low temperatures, or with a Pyrometer for temperatures above 250°C. In the later experiments, a mercury thermometer was also used for temperatures up to 500°C. It was deemed important to know approximately the rate of rise of the temperature of the soils in the autoclave. An arrangement was made by means of a packing valve and electrical resistance thermometers by which this could be done. The rise in temperature of substances placed in the autoclave was relatively rapid at first but reached the maximum slowly. According to these preliminary tests, 35 to 60 minutes, depending on the container used, should be sufficient to thoroughly heat the soil throughout in the autoclave to a temperature of 115°C. at 15 to 20 pounds of pressure in bulks as large as 2 to 3 kgm. One hour and thirty minutes was, however, always allowed for sterilization in the autoclave except where otherwise stated.

In the germination tests the soils were heated in the Petri dishes used in the tests at the lower temperatures, but in clay dishes at the higher temperatures. Fifty grams of air-dry soil was weighed out and used in all cases in the germination tests. As soon as cool after heating, water was added to the soils bringing them all as near as possible up to the same percentage of moisture, which was practically but not quite up to saturation. As soon as all the soils were evenly moistened, 100 seeds, which had previously been counted out, were placed in each Petri dish, merely being scattered over the surface of the soil. The tests were all run in duplicate. The Petri dishes retained a fairly constant supply of moisture from 10 to 12 days, and it was rarely found necessary to add more water to complete the tests. The seeds were picked out with tweezers and the number that germinated at certain intervals, usually of 12, 18, or 24 hours, were recorded. Checks on unheated soil or filter paper were always used.

In the case of heating large quantities of soil with dry heat for plant growth studies, more difficulty was encountered in getting uniformity of heating. In some of the earlier tests the soil was heated directly in the pots used; but later it was found more satisfactory to heat the soil in shallow pans which would

permit of one or two stirrings during the heating process as well as a thorough mixing afterward. After being thoroughly cooled, the soils were watered and sown to seed or seedlings transplanted on the same day or the day following heating. No special attempt was made in the ordinary tests to prevent reinfestation of the pots with organisms.

Extraction of the soil with water was made by allowing equal weights of water and soil to remain in contact for 24 hours with frequent shaking or stirring. The extract was then filtered off through filter paper, usually with reduced pressure when filtering was slow. These extracts were used for germination tests by saturating three or four layers of filter paper in Petri dishes with the solution, using water on filter paper as checks. Extracts obtained in this way were also used for the freezing-point determinations and in the temperature studies with Dewar flasks.

Freezing-point determinations were made with a Beckmann thermometer in the ordinary way, the method differing, however, from that described by Boyoucos (6), in that extracts were used instead of the soil itself. The readings are not intended to represent the actual concentration of the soil solution in the soil as they are no doubt too low, but they do give a fairly good measure of the comparative concentrations of the soil solutions used.

Ammonia determinations were made by the ordinary magnesium oxide distillation method, which again is to be regarded as giving comparative rather than actual amounts of ammonia present.

Seed germination on heated soils

The earlier workers on heated soils did not apparently note any particular effect of heated soil on seed germination. This is to be expected since it is usually only in comparative germination tests that this fact becomes most evident. Stone and Smith (71) were probably the first to carry on experiments along this line. They found an apparent acceleration of germination in heated soils and noted further that different kinds of seeds behaved differently in this respect. Pickering (50) started a fairly extensive study of the subject of the toxic action of heated soils as measured by seed germination. Unfortunately his first papers, especially, are marked by considerable lack of uniformity in results and although most of the general conclusions drawn are quite correct in principle, the data are in many cases meager and confusing. He found, for instance, in one case that heating soil to 100°C. prevented seed germination altogether, while heating to 250°C. had little effect. In reality the opposite is more likely to be the case. On the whole, however, he concludes that the time of incubation increases considerably as the temperature to which the soil is heated is increased, whereas the percentage of seeds germinating decreases. In some cases acceleration was noted on soils heated to 60° or 80°C. Heating to 200° produced the maximum retarding effect. Seeds varied in their response, mustard for instance being more affected by heating than rye. The length of time of heating soil at a certain temperature

had no special effect, but increased moisture content on heating increased the toxicity. Pickering also noted that the toxic property was retained in storage of heated soils, and that its retention was influenced by moisture content and temperature during storage.

In a second paper Pickering (51) reports that the formation of the inhibitory substance to seed germination begins at temperatures as low as 30°C. and reaches its maximum at 250°. He found most soils in their natural state less favorable for germination than water alone, and that sterilization by antiseptics is equivalent to heating to 60° to 75°C. as far as seed germination is concerned. The "poverty" or "richness" of a soil did not seem to bear any relation to its behavior when heated. Takoma soil (obtained from Whitney, U. S. Dept. Agr.) was found to be very toxic when heated and to turn seeds sown upon it black in color. Pickering attempted to show in these and later papers that the toxic property of heated soils was connected with increase in soluble organic matter, but without good success, as the exceptions found were too numerous. His results on the loss of toxicity in heated soils are especially interesting and lead him to conclude that the disappearance of the toxin is due to an oxidation process. Pickering's conclusions on the nature of the toxic substance will be discussed more in detail in a later paragraph.

Russell and his collaborators (62–64) used seed germination tests to a considerable extent as a measure of the changes induced in soils by heating and by treatment with volatile antiseptics. These results are hardly to be compared with that on soils heated to high temperatures such as those used by Pickering (50) and in the investigations reported in this paper. Russell and Petherbridge (64) do not agree, however, with Pickering in the supposition that the toxic agent necessarily disappears in storage or that it is organic in nature.

In making seed germination studies on heated soils, it at once becomes evident that a number of primary factors are concerned, together with a considerable number of secondary factors, which may influence the results secured in such a way as to obscure or exaggerate their importance. The primary factors are especially those of temperature to which the soil is heated, type of soil used, and kind of seed used in the germination test. These factors are all important and fundamental in comparing results. As secondary factors which may be considered as influencing or explaining the results secured, the following have especially been considered: length of time of heating, length of time between heating and the germination test, manner of storage following heating, percentage of moisture at time of heating, method of heating (dry or moist heat), aeration during and after heating, size of soil particles, natural toxicity of the soil, temperature of germination, aeration during germination test, percentage moisture in soil during germination, action of light during germination, size and viability of seeds, and time required for normal germination. Although all these factors could not be studied in detail, they have been given sufficient consideration to indicate the part which they might play in the results secured if they occurred as unavoidable variable factors.

Temperature of heating. The temperature to which a given soil is heated has been found to be probably the most important factor in seed germination tests on heated soils. This is well illustrated in table 2 which shows the relative rate of germination of lettuce seed in Waukesha silt loam soil heated to temperatures ranging from 50° to 350°C. It may be observed that the toxic property of the heated soil begins at 100°C., increases rapidly up to 250°C., and finally decreases at higher temperatures, having practically been lost on soil heated to 350°C. Minor variations sometimes occur which are not shown in this table, i.e., the increased germination at the lower temperatures of heating, (table 6), which condition occurs in certain soils before the temperature of retardation occurs. This is not to be taken, however, as the result of a different type of product forming in the soils heated to the lower temperatures from that produced at a somewhat higher temperature where retardation begins. It is rather to be considered as the stimulating action of a small quantity of the toxic substance produced. It may be, of course, assumed here that the low temperatures destroy the naturally present toxic property of some soils, but reasons for not assuming this to be so will appear later, although most soils do possess a natural element slightly toxic to seed germination. It will also be noted in table 2 that the toxic action is exhibited in this case quite largely by the retarding influence on germination, the final or total germination at the maximum temperature of retardation being as great as that on the not-heated soil. This is not, however, always found to be the case. More frequently, in fact, the total germination at the maximum of retardation is considerably lower than that of the unheated soils, indicating that some of the seeds have not been able to recover from the toxic agent and are in many instances actually killed.

A very considerable number of the tables presented here, as well as a number which are not included, repeatedly show the same general relation of temperatures of heating the soil and the effect on seed germination as stated above. The variation for different soils and different seeds is very great as will be shown by the following considerations. Although the temperature of 250°C. has not been determined with any fine detail as the exact turning point for all soils, its almost invariable occurrence on heating to this temperature as nearly as possible in gas and electric ovens points quite conclusively to the critical temperature for seed germination as lying close to 250°C.

Soil type. The amount of the toxic action produced on heating is very largely dependent upon the soil type used. To illustrate this point, the results shown in table 3 are presented. Seven different soils were used, all heated to 250°C. and their action on germination of lettuce seed compared with the action on unheated checks. A retardation of the germination occurred with all the heated soils used as compared with the checks. The extent of this retardation and the rate of recovery from it is, however, quite different, being greatest and slowest on the Waukesha silt loam and least and most rapid on the fine sandy loam. Although heating to a different temperature, or

using another kind of seed, might have led to results more complicated and more difficult to explain, it is worth while noting here that the extent of the toxic action does not seem to be correlated with any one common characteris-

TABLE 2

Relative rate of germination on soil heated to different temperatures; Waukesha silt loam; lettuce seed

TREATMENT	GERMINATION AFTER						TOTAL
	24 hours	42 hours	66 hours	90 hours	114 hours	138 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated	57	89	92				92
Heated to 50°C.	73	91	94				95
Heated to 100°C.	2	50	80	84	87	90	95
Heated to 150°C.	0	25	71	80	82	85	89
Heated to 200°C.	0	6	32	49	57	67	82
Heated to 250°C.	0	0	3	15	27	35	92
Heated to 300°C.	0	77	90				95
Heated to 350°C.	41	92	95				95

TABLE 3

Relative rate of germination of seed on different soils heated to 250°C.; lettuce seed

SOIL	TREATMENT	GERMINATION AFTER						TOTAL
		24 hours	42 hours	66 hours	90 hours	114 hours	138 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Peat	Check	68	85	87	89			89
	Heated	0	0	1	5	16	62	84
Muck	Check	66	83	85	87			87
	Heated	0	0	0	12	69	83	90
Waukesha silt loam	Check	73	88	89	90			91
	Heated	0	2	3	7	15	30	67
Virgin sandy loam	Check	78	94	95				95
	Heated	0	0	1	36	62	72	85
Fine sandy loam	Check	75	87	88				88
	Heated	0	1	7	80	95	96	98
Clay	Check	70	81	83	86	87		87
	Heated	0	4	7	45	60	66	84
Norfolk sand	Check	77	87	88				88
	Heated	0	4	8	46	69	82	92

tic of the soils used. Statements have been made, and also contradicted, in the literature that the amount of the toxic action is in proportion to the amount of vegetable matter or humus present in the soil, or that it is corre-

lated with soil fertility. That the amount of organic matter in a soil plays a large part, and that the toxic property is formed from organic matter in one form or another is seemingly quite evident. In an attempt to illustrate this, the results shown in table 4 were secured where different amounts of quartz

TABLE 4

Influence of content of vegetable matter on the injurious action to seed germination on heating to 250°C.; cabbage seed

PERCENTAGE VEGETABLE MATTER	TREATMENT	GERMINATION AFTER 24 hours	42 hours	66 hours	90 hours	114 hours	138 hours	TOTAL
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ground quartz	None	10	58	72	75			75
	Heated	28	60	68	75			75
Quartz with 10 per cent peat	None	35	62	77	81			81
	Heated	4	19	68	79			80
Quartz with 20 per cent peat	None	40	74	79	81	82		82
	Heated	0	3	7	30	53	54	54
Quartz with 50 per cent peat	None	34	63	68	73	76		76
	Heated	0	0	0	10	32	47	47
Peat	None	34	70	77	79	80		80
	Heated	0	0	0	0	1	2	2
Quartz with 20 per cent manure	None	30	62	66	69	70		70
	Heated	1	21	50	64	71		71

TABLE 5

Loss in weight of soils on heating to 250°C. and on ignition

SOIL	LOSS ON HEATING TO 250°C.	LOSS ON IGNITION	ORGANIC MATTER LOST ON HEATING TO 250°C.
	per cent	*per cent*	*per cent*
Peat	5.4	68.1	7.8
Muck	1.4	14.8	9.4
Waukesha silt loam	0.83	5.4	15.3
Virgin sandy loam	0.76	5.6	13.5
Fine sandy loam	0.55	4.3	12.7
Red clay	0.43	5.5	7.7
Norfolk sand	0.35	1.9	18.4

sand and well decomposed peat were mixed and heated, and the toxicity was found to be in a large measure proportional to the amount of organic matter present. This, however, cannot be considered to approach conditions present in normal soils with varying percentages of organic matter. Table 5 shows the percentage loss of organic matter in soils heated to 250°C. as compared

with the total organic matter present as measured by the loss on ignition together with the per cent of the organic matter lost on heating to 250°C. Although the data at hand upon this subject are not sufficient to be significant, it is worth noting that the percentage of matter lost on ignition for the most toxic and the least toxic soil are too close to warrant any degree of importance being attached to them. It appears from these results, however, that peat and muck though highest in organic matter and losing the greatest weight on heating to 250°C. did not lose as large a percentage of their organic matter content as the others and were not as toxic as Waukesha silt loam which had considerable less organic matter but lost proportionately a larger amount on heating to 250°C. The indications are, therefore, that the amount of toxicity produced on heating is not proportional to its organic matter, but that it is rather proportional to the state of the organic matter or to the percentage which is actually decomposed but not taken care of by the soil itself through its powers of absorption. These probabilities will be considered more in detail under a study of the nature of the toxic substance.

That results may be quite different when lower temperatures and different seeds are used, may be seen by referring to table 6. Heating to 50°C. stimulated cabbage seed germinations on most soils. Heating to 100°C. sometimes stimulated and at other times retarded germination. Heating to 250°C. retarded germination in all cases; while heating to 500°C. brought the soil back practically to normal in all cases as far as toxicity is concerned. Heating to 800°C. either stimulated germination, or heating to such a high temperature may be regarded as having merely eliminated the inhibiting elements naturally present in soils. The difference in results at the lower temperatures from that at higher temperatures has already been explained as being believed to be due to the stimulating action of a small amount of the toxic substance. This stimulating action may be considered to be exaggerated in table 6 on account of the use of cabbage seed in the germination test which is more "resistant" to the toxic substance than lettuce seed, as will be shown in the following paragraphs. If wheat seed had been used the acceleration would have been more marked. The Waukesha silt loam soil being found to be most toxic to seed germination when heated, has been used most in the toxicity experiments.

Influence of type of seed used on results secured. The great variation in susceptibility of seeds of different species of plants to the toxic action of heated soils opens two lines of interesting inquiry: the first being as to the nature of the toxic substance produced when the soil is heated, and the other being the causes of these differences in "susceptibility." The former is the one of most concern in the problems undertaken. The latter is a physiological problem often encountered in dealing with the relation of various chemicals to plant tissues, and may have an important bearing in explaining the nature of the toxic agents in heated soils. If a correlation could be established between variations in "susceptibility" of different seeds to heated soils with that of the

TABLE 6

Effect of heating various soils to different temperatures on germination of cabbage seed

SOIL	HEATED TO	GERMINATION AFTER 48 hours	72 hours	96 hours	120 hours	144 hours	Total
	°C.	per cent	per cent	per cent	per cent	per cent	per cent
Peat*	Check	71	79	84			84
	50	76	80	82	83		84
	100	50	80	85	88		89
	250	21	32	40	53	65	77
	500	85	93	95			96
	800	83	86	89	93		93
Sparta sand	Check	77	87	90	92	93	93
	50	92	94	94	95		95
	100	81	90	91	92	93	93
	250	40	60	66	68	70	80
	500	73	81	86	90		90
	800	86	91	91	93		94
Red clay	Check	80	88	90			90
	50	73	78	81	82		83
	100	79	89	89	91	92	92
	250	30	47	54	57	67	92
	500	79	84	86	88	89	89
	800	79	85	87	88		91
Waukesha silt loam	Check	72	87	89			90
	50	74	84	88			88
	100	7	17	24	31	32	83
	250	4	7	8	11	13	81
	500	72	79	84	85		86
	800	79	83	87	90		90
Fine sandy loam	Check	71	80	80			80
	50	89	92	95			97
	100	91	96	98	99		99
	250	63	72	81	83		86
	500	84	87	89	90		92
	800	79	85	88	90		90
Miami silt loam	Check	77	87	89	91		91
	50	86	89	93	94		94
	100	63	86	92	93	94	96
	250	47	68	77	83	86	95
	500	71	78	80	81	84	85
	800	83	87	90	90	93	93

* An undecomposed peat, not the same as described in table 1.

toxic action of certain pure chemicals on the assumption that seeds react differently to various chemicals, good indirect evidence of the nature of the toxic substance could be established.

The reaction of seeds to chemicals or to heated soils may be either in the direction of stimulated or retarded rate of germination, or in increased or decreased total percentage germination. The experiments are, however, confined to and based mostly upon the effect on rate of germination. If we select seeds of a wide range of families of cultivated plants as is shown in table 7 and compare the germination on soil heated to 250°C. with the germination on unheated soil, it will be found that on the particular soil used, the seeds of all families are retarded in germination. It can be noted, however, that the seeds differ very markedly in their resistance to the toxic property, as indecated by the relative germination at a certain period. The rapid recovery from the toxic action by some of the species and the final increased percentage of germination of certain species above that on unheated soils is notable. The seeds of wheat and cucumber are seen to belong to the very "resistant" class, whereas those of clover and lettuce are very "susceptible" to the toxic property. Various gradations between the most and least susceptible species occur, and it is reasonable to suppose that other seeds more resistant and more susceptible than those tested here exist in nature. Working with a soil becoming very toxic on heating to the temperature most productive of toxicity (i.e., 250°C.), the advantage in simplifying the facts, over that on heating this soil to a lower temperature, is to be seen in table 8, where Waukesha silt loam was heated to 115°C. and sown to different seeds. In this case, we have no retardation but stimulation of the most resistant species together with marked retardation of the "susceptible" species, and it becomes easier to misconstrue the nature of the toxic properties present.

Various factors of secondary importance influencing germination on heated soil. Turning to some of the other factors mentioned which deserve consideration as influencing or explaining the results noted above, it may be well to consider them briefly at this point, illustrating by data only those which may be especially important or interesting. In all experiments on seed germination and plant growth the seed has usually been sown as soon as possible after heating the soil, this time rarely exceeding 6 hours, and where delayed has been usually due to difficulty in wetting certain of the soils uniformly after heated to a low temperature. Naturally, however, the question arises as to what may happen in soils stored under different conditions for a considerable period of time between heating and seeding. All experiments of this nature have gone to show that the toxic property remains practically unchanged if the soil is kept in a dry condition, but that if the heated soil is kept in a moistened condition for a considerable period of time, changes occur and the toxic property is gradually lost or destroyed. This point is illustrated in table 9 where two different soils heated to 250°C. were kept for three months in the moist and dry condition, the toxicity as shown by seed germination being entirely lost in the

TABLE 7

Relative rate of germination of different seeds on soil heated to 250°C.; Waukesha silt loam

SEED	SOIL TREATMENT	GERMINATION AFTER							TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	162 hours	186 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Garden cress......	Check	26	34	44	62	67	68		68
	Heated	0	1	1	2	3	5	15	24*
Wheat............	Check	36	76	86	93				93
	Heated	11	68	87	94	95			96
Lettuce...........	Check	69	72	73	83	85	88		88
	Heated	0	0	1	2	4	7	27	50*
Clover............	Check	44	68	79	85	86			86
	Heated	0	3	3	5	7	9	37	68*
Flax..............	Check	96	97	98					98
	Heated	21	57	71	86	90	92	95	95
Cucumber.........	Check	41	65	75	84	85	86	87	88
	Heated	23	54	68	82	84	85	87	88
Cabbage..........	Check	33	53	59	65	65	66		66
	Heated	0	0	1	3	11	29	51	56*
Onion............	Check	3	16	45	73	78	78	80	80
	Heated	0	1	8	54	70	73	75	75
Spinach...........	Check		5	15	37	48	51	57	59*
	Heated		0	1	5	13	20	36	47*
Buckwheat........	Check			8	20	29	46	61	63
	Heated			1	12	22	37	60	64
Carrot............	Check			43	59	64	67		67
	Heated			0	2	15	42	63	68
Tomato...........	Check				30	54	58	65	67
	Heated				0	1	10	53	61
Phlox.............	Check				7	11	13	20	24
	Heated				0	0	0	3	11*
Snapdragon.......	Check				6	31	41	47	49
	Heated				0	0	0	1	8*
Cotton...........	Check				5	14	39	72	74
	Heated				0	1	11	65	73
Linaria..........	Check					17	39	58	67*
	Heated					0	0	0	2*
Parsnip..........	Check						4	12	17*
	Heated						0	4	8*

* Seeds moldy and failure to show a greater total germination may be partially due to this.

moist state but quite largely maintained in the dry state. This persistence of the toxic agent is further shown in table 10 where heated soil kept dry for two years still showed a very considerable toxic action. That aeration has no particular influence in this regard where soil is stored dry but that it has

TABLE 8

Relative rate of germination of different seeds on heated soil. Waukesha silt loam heated to 115°C.

SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	258 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Clover	None	65	81	85	86	88		88
	Heated	57	74	80	84	87	88	88
Cabbage	None	56	83	88	90	91	92	92
	Heated	69	82	87	88	89	90	91
Garden cress	None	20	48	64	68	71	72	73
	Heated	16	40	52	56	60	66	66
Onion	None	2	67	93	95	96	97	97
	Heated	4	64	93	96	97		97
Buckwheat	None		7	14	23	33	55	55
	Heated		3	14	21	29	42	42
Tomato	None		9	84	96	97	98	98
	Heated		2	69	91	98	99	99
Carrot	None		10	36	53	58	64	64
	Heated		3	24	50	62	67	67
Lettuce	None	83	92	94				94
	Heated	8	40	67	83			83
Flax	None	97	98					98
	Heated	92	94	95				95
Wheat	None	30	93	97				97
	Heated	47	96	97				97
Cucumber	None	5	65	82	84	85		86
	Heated	10	71	86	87	88		88

some influence where soil is stored moist, is shown in table 11, in which experiment, soils were stored in Mason jars with covers sealed on in one case, but left off in others. No consideration was given to reinfestation with microorganisms in this experiment. This test indicates that the time of seeding following sterilization or heating has little influence if the soil is kept

TABLE 9

Effect of storage of heated soil for 3 months in moist and dry condition upon rate of seed germination; cabbage seed

SOIL	TREATMENT	STORED	GERMINATION AFTER 43 hours	55 hours	67 hours	79 hours	97 hours	TOTAL
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Waukesha silt loam	Check	Dry	49	53	70	73	73	75
		Moist	53	65	70	74	76	78
	Heated to 250°C.	Dry	13	25	38	50	60	72
		Moist	50	61	66	71	73	75
Fine sandy loam	Check	Dry	43	53	62	66	66	66
		Moist	45	55	62	64	66	68
	Heated to 250°C.	Dry	19	39	50	61	64	70
		Moist	49	65	68	73	76	78

TABLE 10

Influence of two years storage of dry heated soil on relative rate of seed germination; Waukesha silt loam; clover seed

SOIL TREATMENT	STORED DRY	GERMINATION AFTER (PERIOD) 1	2	3	4	5	6	TOTAL
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated................	0	41	80	86				86
Heated to 250°C...........	0	0	2	4	7	11	14	53
Not heated................	2 years	20	75	81	83	85	87	87
Heated to 250°C...........	2 years	0	11	22	42	66	70	75

TABLE 11

Influence of moisture and aeration of heated soil during storage on germination of seed; Waukesha silt loam; lettuce seed

TREATMENT	STORAGE Water	Air	GERMINATION AFTER 24 hours	36 hours	48 hours	60 hours	72 hours	84 hours	TOTAL
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated...........	+	+	75	95	96				96
		−	26	87	91	92			92
	−	+	3	62	89	92			92
		−	11	76	87	91			91
Heated to 250°C......	+	+	0	10	58	73	74	74	
		−	0	2	19	48	51	52	
	−	+	0	1	1	2	8	9	
		−	0	0	0	2	4	5	

dry, but that the toxicity is gradually lost where heated soils are kept moist and exposed to aeration and contamination. It points further to the fact that some of the toxicity to seed germination may be diminished toward the close of a seed germination test in Petri dishes, and also that a part of this, which if given off from the soil rather than destroyed in it, may be partially retained in the enclosed chamber due to lack of good aeration, and hence exaggerate the retardation which fact may partly explain the more marked retardation under these conditions than is ordinarily observed on heated soils under natural conditions.

That the amount of moisture present in the soil at the time of heating may influence the amount of toxic substance formed or its retention by the soil is shown in table 12. Here 50 gm. of Waukesha silt loam were placed in Petri dishes and varying amounts of water added before heating in the autoclave, after which it was made up to equal percentages of water and seeded to lettuce.

TABLE 12

Relative rate of germination of seed on soil heated with different amounts of moisture; Waukesha silt loam; lettuce seed

TREATMENT	WATER ADDED TO 50 GMS. AIR-DRY SOIL	GERMINATION AFTER					TOTAL
		30 hours	42 hours	54 hours	66 hours	78 hours	
	cc.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated		76	89	91	94	95	96
Heated 115°C.	0	11	29	68	87	89	93
	5	17	49	79	89	92	92
	10	10	27	71	87	91	92
	15	6	23	63	82	85	89
	20	4	19	61	80	87	94
	25*	2	16	52	80	85	90

* Saturated.

Increased water content at the time of sterilization apparently increases the toxic action. There is also, however, an indication that adding a small percentage of water may be less productive of injurious products than heating an air dry soil, although this may be associated with physical changes, since the ability of relatively dry soil heated to 115°C. or thereabouts, to absorb moisture is very poor. Throughout the experiments, the soils used for heating have been usually air dried, this of course, being advantageous in heating to higher temperatures with dry heat.

As to time of heating, an arbitrary standard must be chosen and maintained as closely as possible, the soil being allowed to become gradually and as uniformly heated to the desired temperature as possible within that period of time. Where large quantities of soil are heated it is important to mix the heated soil thoroughly before taking fractions for tests. To determine the effect of length of time of heating a soil on seed germination, the Waukesha silt loam was heated for different lengths of time, ranging from 10 to 160 minutes, in the

autoclave at 115°C. (table 29). The soils were spread out in a thin layer to make thorough heating at the short periods of time possible. Ammonia determinations and freezing point determinations of the soil solution were also made in this experiment. The differences due to length of time of heating at this temperature were small and perhaps insignificant, but there is seemingly a slight increase on increased time of heating followed by a lowering of toxicity to seed germination which then remains fairly constant and although changes do occur they do not seem to be constantly in one direction. This was seemingly not correlated with ammonia content or concentration of the soil solution. Similar rise and fall of toxicity was also obtained for soil heated to 200°C. for 2, 4, 6, 8, 10, and 12 hours, but no decisive conclusion could be drawn as to the influence of time of heating on seed germination.

TABLE 13

Effect of temperature of germination on toxic action of heated soil; Waukesha silt loam; red clover

SOIL TREATMENT	TEMPERATURE OF GERMINATION	GERMINATION AFTER					TOTAL
		48 hours	72 hours	96 hours	120 hours	144 hours	
	°C.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	12	5	40	68	80	81	82
	15	14	52	67	73	76	79
	21	39	79	90	91	92	92
	24	77	88	91	91	91	91
	26	75	86	86	87	87	87
	35	27	37	44	56	60	
Heated to 250°C.	12	0	0	2	9	19	77
	15	0	1	5	10	19	85
	21	0	5	12	20	25	83
	24	6	15	21	40	67	71
	26	2	3	5	27	58	60
	35	0	0	0	0	1	

To determine the relation of the temperature at which the germination test is made upon the degree of toxicity shown by heated soils, the results shown in table 13 were secured. Waukesha silt loam soil was used, and the heated and unheated checks were placed at different temperatures in a series of Altmann and other electrically regulated incubators where constant temperatures could be maintained. The optimum temperature for the germination of clover seed was found to be close to 24–26°C. The same temperature apparently held for germination in both heated and unheated soils. The proportion of germination seemingly holds the same for all temperatures on both heated and unheated soils excepting at the highest temperature, i.e., 35°C. where the injurious action of the combination of high temperature and soil toxicity is proportionally greater for the heated soil. It is apparent, however, that a fairly large range of temperature variations will not materi-

ally effect results in the germination experiments. No special efforts were, therefore, made to keep all germination tests at constant temperatures, and usually only room temperatures (about 20–22°C.) were used. These were kept quite constant day and night, however, by the automatic regulation of the steam-heating system.

In the preceding temperature test of germination, lettuce seed was used in the first trial. It was found that the lettuce seed germinated very poorly in all the dishes in the incubators which excluded light. It was at first thought that poor seed had been used but seed from the same samples in the laboratory germinated normally. It was, therefore, concluded that light must be a controlling factor in lettuce seed germination, and this was found to be the case. It has, of course, been repeatedly shown that light is necessary for normal germination in many seeds (38). As lettuce seed was most commonly

TABLE 14

Influence of absence of light on the germination of lettuce seed in heated and unheated soil; Waukesha silt loam

SOIL TREATMENT	LIGHT	GERMINATION AFTER					TOTAL
		30 hours	48 hours	72 hours	96 hours	120 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	+	75	88	90	93	95	97
	−	1	7	8	9	10	10
Heated to 115°C.	+	12	40	62	74	84	90
	−	3	4	5	5	6	8
None	+	82	93	93	94	95	96
	−	0	10	16	20	21	22
Heated to 250°C.	+	0	2	8	15	23	26
	−	0	3	7	8	12	13

used in the tests in my experiments, however, on account of their susceptibility to the toxic action of heated soils and their ease and convenience in handling, it was found necessary to keep the seeds germinating in the open room rather than in a dark incubator. An interesting fact noted in this connection (table 14) was that the combined action of absence of light and of soil toxicity did not retard seed germination in proportion to the expectations when compared with unheated soils in the absence of light. In other words, the heated soils apparently supplied in some small measure a substitute for light in germination. This is especially interesting considering the results of Gassner's work (20) in replacing the effect of light with certain nitrogenous salts.

In an effort to determine the relation of size of soil particles used to germination on heated soils, a heated sample was screened to different sized

particles and sowed to lettuce. No particular conclusion is warranted from this test; although there is an indication that the larger-sized particles are less toxic than the finer grades. It is possible, however, that other factors than toxicity play a part here. The results are in agreement with those of Stone and Monahan (70) on this subject, and as suggested by them the precaution should be taken of using soils of a standard degree of fineness for germination tests. The addition of ammonia to soils of coarse and fine textures indicated more markedly that greater toxicity occurred in soils of fine than in those of coarse particles.

Pickering (50) has shown that moisture content of soil in germination tests may vary quite widely without greatly influencing the results. This is in accordance with results obtained in an experiment carried on by the writer in this connection. Too low moisture content reduces, of course, the rate of germination, while the higher moisture contents tend to increase rate of germination, but probably reduce total germination slightly. It has already been mentioned that certain soils heated to low temperatures take up water with great difficulty. The loss of capillarity is in some instances very striking, the soil seeming in fact at times to have lost all affinity for water. In "kneading" the soil up with water in order to get some degree of uniformity of moisture throughout, it is possible that a physical condition is obtained unfavorable to germination quite apart from toxic action. This influence, however, has not been deemed sufficiently important in this connection to warrant detailed study. Soils heated to high temperatures do take up water very much more readily than unheated soils, and it was found, for instance, that a virgin sandy loam heated to 250°C. took up water two to three times as fast as the unheated, while this soil heated to 115°C. took up water only one-half to one-eighth as fast as the unheated soil.

The size of the seed is not considered as influencing the susceptibility to the toxic action of the heated soil. Corn and peas, for instance, being larger than rye or buckwheat, are more susceptible, while clover and lettuce being smaller than the latter, were also more susceptible. Similarly the gross chemical composition, as for instance starchiness and oiliness, does not seem to be in any way connected with susceptibility or resistance to injury. Sweet corn, for example, is only slightly more susceptible than field corn, and flax and wheat are about equally resistant. Age, or maturity of the seed, as far as noted does not apparently influence results to any extent. It seems most probable that the factor which determines the nature of the response in seed germination lies in the selective permeability of the seed coats to certain chemical substances. With the above facts in mind tests were begun with the idea of determining and measuring more closely the nature of the toxic substances in heated soils. Before taking this up it is probably advisable to consider how far these facts concerning seed germination are correlated with plant growth on heated soils.

Plant growth on heated soils

Although the beneficial action of heated soils on the growth of the higher plants had been known for a long time, it was apparently not until about the middle of the last century that any serious experimental investigation was undertaken in this field. Struckman (73) in 1857 in addition to other lines of work on burned soils obtained good results with cabbage and rape, but noted no especially good results with vetch and flax on burned soil. The experiments of Fleischer (17) and his associates at the "Moor-Versuchs Stationen" were continued for a number of years, heated soils giving on the whole good results with plant growth, although the economy of burning was open to question. With respect to the influence of dry heat on soil, the more recent investigations along this line are especially those of Pickering (53), Seaver and Clark (68), Fletcher (18), and Kelley and McGeorge (30). A more extensive amount of literature exists upon plant growth in steamed soils, and there is a great deal of evidence showing the beneficial action of steamed soils on plant growth since the time of Franke (19), Pfeiffer and Franke (49), and Kruger and Schneidewind (37). Studies on the injurious action of heated soils began especially with the results of Deitrich (13), and these have since become a very important part of the investigations upon the subject. Following the work of Girard (21) who applied carbon bisulfide to the soil used for the growth of several agricultural crops, a large amount of literature has accumulated with the object of explaining the beneficial action on plant growth of antiseptics applied to soils. That sterilization by chemical agents and by heat have one and the same effect on soil as far as plant growth is concerned has been generally accepted, and the two methods, sterilization, or "partial sterilization" by heat or chemicals are, therefore, from a biological standpoint, usually considered together by most authors. The theories concerning the causes of the modification of plant growth on sterilized soils have already been briefly mentioned, and the changes in and caused by sterilized soils outlined. The experimental results presented here are mainly concerned with establishing further evidence relative to the toxic and beneficial action of heated soils, and the immediate and subsequent changes produced on different soils as measured by the growth of different plants, with the purpose of arriving at a better conception of the nature of the beneficial and toxic substances produced in heated soils. Experiments were therefore undertaken to determine the influence of the following as measured by plant growth: (a) the response of different types of soil to sterilization; (b) the effect of different temperatures of heating; (c) the effect on different kinds of plants; (d) the influence of different lengths of time of heating; (e) the influence of repeated heating; (f) the influence of various soil environmental factors on the expression of the injurious and beneficial action.

Soil type. The remarkable variation in results which may be secured with plant growth on different soils when sterilized is best illustrated by table 15. As wide a variety of soil substances as could be secured were used including those previously described, and also pure leaf mold, greenhouse compost, and a very acid sand (lime requirement 9.38 tons per acre). After being sterilized at about 110°C. these soils together with their checks were planted to tomatoes. A wide variation in results was noted from the start, both in quantitative and "qualitative" response, and these variations and changes continued to occur throughout the period the plants were allowed to grow. Comparing the yield of the different unsterilized soils it may be noted that the compost was apparently the most fertile and the red clay the least fertile for tomatoes. The greatest percentage increase in yield, however, occurred on the muck soil 275 per cent and the greatest reduced yield on the virgin sandy loam where the plants were practically all killed (plate 1, fig. 1). There was manifestly no correlation between the fertility of the soil and the toxic or beneficial action. Considering total organic matter, in those soils in which the loss by ignition has been determined (table 1) it may be seen that peat, highest in organic matter (68.1 per cent), though very toxic on being sterilized, was not so much so as virgin sandy loam with only approximately 5.6 per cent organic matter. Muck with about 14.8 per cent organic matter was only slightly more beneficially influenced than Miami silt loam with only about 5.4 per cent organic matter. There is, therefore, also apparently a lack of correlation between the behaviour of the heated soils toward plant growth and their content of organic matter. That fertility and organic matter bear a close relation to the action on plant growth upon sterilizing the soil, has been repeatedly suggested by various writers but frequently disproven when subjected to experimental test. That the organic matter should bear some relation to the toxic and beneficial action upon heated soils is, however, too evident in connection with other facts to dismiss the matter without further consideration. The influence of the organic matter may be indirect and complicated by various factors, especially by its relation to the absorptive capacity of the soil, its chemical state as influencing extent of decomposition upon heating, and by its relation to bacterial activity subsequent to sterilization. The relation of absorptive capacity, especially, will be considered in connection with the discussion of the nature of the toxic agent. For the present we can only conclude that very little is suggested by the gross character of the soil itself as to its action upon plant growth when subjected to sterilization by heat.

Aside from simple retardation and acceleration of growth, several other interesting results were obtained where tomatoes were grown in different soils heated to about 110°C. These are indicated briefly in table 15 but will be discussed in more detail under the subject of the "qualitative" action of heated soils. The extreme toxicity of virgin sandy loam to tomatoes when sterilized should not, however, be allowed to confuse the problem being pri-

TABLE 15

Air-dry weight of tomato vines grown on different soils sterilized at 110°C.*

SOIL	TREATMENT	DRY WEIGHT	PER CENT INCREASE (+) DECREASE (−)	EARLY GROWTH	LATE GROWTH
		gm.			
Miami silt loam......	None	1.60			Leaves yellowing
	Sterilized	5.65	+254	Leaves mottled; slight retardation	Leaves a healthy green
Muck.......	None	1.60			Leaves yellowing
	Sterilized	6.00	+275	No retardation	Leaves a healthy green
Fine sandy loam......	None	1.45			
	Sterilized	3.05	+110	Slight retardation	
Norfolk sand.	None	0.50			
	Sterilized	0.90	+ 80	Purple pigmentation	
Leaf mold....	None	6.20		Purple pigmentation	
	Sterilized	6.95	+ 12	Leaves mottled; slight retardation	
Red clay.....	None	0.15		Purple pigmentation	
	Sterilized	0.15	0	Purple pigmentation; no retardation	
Peat.........	None	0.90			
	Sterilized	0.30	−200	Marked retardation; purple pigmentation	Change in pigmentation and recovery started
Waukesha silt loam......	None	4.60			
	Sterilized	2.10	−119	Uneven retardation	Marked toxic action on some
Acid sand....	None	0.85		Purple pigmentation	
	Sterilized	0.35	−142	Purple pigmentation; slight retardation	Several spots and lesions on leaves and stems
Greenhouse compost...	None	9.65			
	Sterilized	5.35	− 80	Leaves mottled; slight retardation	Toxic action started on leaves
Virgin sandy loam......	None	5.60			
	Sterilized	0.15		Marked retardation; lesions on leaves	Plants practically killed by lesions on leaves and stems

* Six plants—62 days' growth in duplicate pots.

marily considered, i.e., simple retardation. This extreme toxic action may not be due to the same cause as that of simple retardation. The percentage decrease on the virgin sandy loam is not included in the data given. This type of injury in which plant tissue is actually destroyed, has been termed "chemical" injury in order to distinguish it from retardation where no such injury appears to occur. That "chemical" injury is not confined to virgin sandy loam soil alone, however, is evidenced by the same but less marked occurrences in three of the other soils used. The results secured in table 15 would have been quite different if other plants had been used, as will be shown later. On the other hand, tomatoes are not the only plants subject to forms of "chemical" injury. Furthermore the temperature to which the soil is heated, the length of time of heating, and the temperature of the soil during the growing period are all influential in determining the occurrence and extent of injury resulting. The results presented in table 15 only show, therefore, what may occur under a certain set of conditions when various types of soil are used. It should also be noted that the results as shown by the final yields are only comparative for that stage of plant growth at which the crops were harvested. It requires a longer period for some soils or plants to recover from the toxic action than others, and in certain instances the greater the toxic action and the time required for recovery, the more pronounced the subsequent increase. The yields should preferably be taken therefore at the maturity of the plants. In experiments under greenhouse conditions this is frequently impossible with certain crops and is not regarded as essential with others. The yields given are therefore in terms of dry vegetative matter produced during a certain period of time regardless of maturity but usually at a stage when conditions similar to that which may be expected at maturity have been reached. Percentages of increase or decrease in yields, therefore, may not indicate the maximum of either but rather the comparative yields at a certain time. On the other hand it is possible so to regulate the time of taking the yield, by noting the stage and vigor of recent growth, that any serious error in this respect may be avoided.

In connection with the studies on influence of soil type, an experiment was conducted to determine whether any difference existed between surface soil and subsoil in their behavior toward sterilization. Stone and Monahan (70) convey the impression that such a difference exists owing to the location of the soils rather than to their chemical or physical characteristics, although it is probable that the authors did not intend to convey such an impression. These writers report a decrease in yield of soy beans in sterilized subsoil as compared with sterilized surface soil. These results could not be corroborated. With soy beans a Miami silt loam surface soil showed an increase of 91.3 per cent and the subsoil an increase of 118.7 per cent over the unsterilized soil. Virgin sandy loam surface soil showed a decrease of 20 per cent for the surface soil and an increase of 58.3 per cent for the subsoil. These tests

though not carried out in detail, indicate that nothing definite can be concluded from the location of the soil with reference to the surface as to its action to plant growth upon sterilization. The results are, on the other hand, not correlated with content of organic matter in the soil as suggested by Stone and Monahan's conclusions.

Temperature of heating. The temperature to which a soil is heated has a marked influence upon its behavior toward plant growth as is illustrated by a preliminary experiment (table 16) where cabbage was used on two different soils heated to 115°, 250°, and 350°C. The early plant growth was retarded to the greatest extent on both soils on heating to 250°C., but the subsequent beneficial action is seen to be the greatest at this temperature of heating. The muck soil gave a decidedly greater beneficial action on heating than did the Waukesha silt loam, but it is not to be expected that all soils will show beneficial action at a given time after heating to 250°C., nor that all plants will

TABLE 16

Dry weight of cabbage produced in soil on heating to different temperatures

TEMPERATURE OF HEATING	MUCK SOIL		WAUKESHA SILT LOAM	
	Weight	Increase	Weight	Increase.
°C.	gm.	per cent	gm.	per cent
Check	1.95		2.55	
115	8.60	340.7	3.40	33.3
250	9.70	402.5	4.95	94.1
350	4.35	123.0	3.60	41.1

respond in the same manner. On heating six different soils to 250°C. the increase in yield of tobacco, for example, ranged as follows: Muck, 571 per cent; Waukesha silt loam, 473 per cent; clay, 150 per cent; fine sandy loam, 96 per cent; virgin sandy loam, 62 per cent, while the peat soil showed a decrease of 25 per cent. The retarding influence of the peat, however, would have been represented by a much greater figure earlier in the test, while, on the other hand, at the time of cutting the plants they were growing vigorously, indicating that in two or three weeks the yield on the heated peat would have exceeded that on most of the other heated soils. Such seeming discrepancies in results do not alter the fact, however, that there exists a maximum degree of toxicity followed usually by a maximum degree of beneficial action to plant growth on soils heated to approximately 250°C. That this critical temperature lies closer to 250°C. than to 200° or 300°C. has been repeatedly noted and is illustrated in table 17 where the relative growth at the end of one month (placing the largest growth at 100), together with the final yield is shown. In a similar manner the retarding action of virgin sandy loam to tomatoes is shown in plate 1, figure 2 and the benefits of heated muck soil on radish is shown in plate 1, figure 3. The decreased yield (table 17)

on heating to 50°C. is believed to be due to loss of nitrates or other forms of nitrogen on drying, together with no beneficial action in the way of increased availability of plant food, due to heating at this low temperature. While tobacco recovered relatively rapidly from the toxic action, other plants may require a relatively much longer time (plate 2, fig. 1), and may even in some instances entirely fail to recover during the normal life of one annual plant, although such "recovery" of the soil may be shown in the later crops. The time required for recovery is, usually, in proportion to the degree of toxicity; consequently we find that on soils heated to different temperatures the earliest growth is usually best on those soils not heated or on soils heated to 350°C. or above, where no toxic principle is present. If the toxicity at 100°C. is relatively small a rapid recovery occurs and in the space of two weeks to a month it will usually be found that the plants on soils heated to this temperature are most vigorous owing to the beneficial action obtained.

TABLE 17

Influence of heating soil to different temperatures on yield of tobacco; Waukesha silt loam

TEMPERATURE OF HEATING	RELATIVE GROWTH AT END OF MONTH (ESTIMATED)	DRY WEIGHT	INCREASE
°C.		gm.	per cent
Check	95	1.55	
50	60	1.15	—34
100	100	2.70	74
150	90	4.85	212
200	85	5.20	235
250	75	6.40	312
300	85	5.90	280
350	95	4.05	161

At this time, however, the plant growth at the 250°C. temperature will usually be the poorest with less retarding action at the 200° and 300° temperatures. Recovery then follows at 150°C., 300°C., 200°C., and finally at 250°C. Once the injurious action is lost, the increase in plant growth occurs relatively rapidly and at a time when the soils heated to the lower temperatures seem to have exhausted the beneficial property produced, the soils heated to 200° and 250°C., may be at their height of vigor of growth. The apparent correlation between the action of soils heated to different temperatures on plant growth and their action on seed germination is evident, and seemingly indicates that the toxic principle is the same in both cases.

Results vary with kind of plant grown. The extreme toxicity of heated virgin sandy loam soil to tomatoes has been shown. If now, different types of plants are grown in this soil, heated to the same temperatures (table 18), it is found that certain plants, in fact most plants, thrive on this heated soil which is so toxic to tomatoes as to practically kill them in many instances (plate 2, fig. 2). Buckwheat and wheat show an increased growth of 321.8

per cent and 245.9 per cent, respectively, while tomatoes show a decrease of 41.7 per cent (table 18). This experiment was conducted during the summer and the decrease is considerably less than that which may be expected to occur during cooler seasons of the year, as will be shown later. That this difference in "resistance" and "susceptibility" of plants to the toxic action of heated soils is characteristic on all soils and with all plants cannot be doubted although no attempt has been made to prove this point. The results obtained at any one time are always complicated by a considerable number of factors, of which probably the most important is environment, and consequently the results are likely to be confusing unless properly analyzed. The

TABLE 18

Effect of sterilization of soil at 115°C., in autoclave on yield of different crops; virgin sandy loam

CROP	TREATMENT	DRY WEIGHT	INCREASE	REMARKS
		gm.	*per cent*	
Buckwheat	None	3.20		
	Heated	13.50	321.8	Early growth markedly retarded
Radish	None	2.15		
	Heated	8.45	293.0	Early growth retarded
Soy beans	None	8.10		
	Heated	8.05	0	Early growth retarded. Foliage shows "chemical" injury
Wheat	None	1.85		
	Heated	6.50	245.9	Early grow retarded
Lettuce	None	0.80		
	Heated	1.60	100.0	Early growth retarded
Tomatoes	None	1.15		
	Heated	0.67	−41.7	Early growth retarded. Marked "chemical" injury

results of one other experiment may be cited, however, in this connection in which three different soils heated to 100°C. were grown to four different crops. Muck soil gave an increase of 140.9 per cent for radish as compared with 40.6 per cent for lettuce. Heated fine sandy loam increased the yield of wheat 119.1 per cent, but decreased the yield of lettuce 106.6 per cent, and virgin sandy loam increased the yield of radish 1.7 per cent as compared with an increase in this case of 76.4 per cent for tomatoes. These results illustrate further the marked variation in the response of plants to sterilized soils. Other results of this nature have shown in general that the Gramineae as a whole are relatively resistant to the toxic action and consequently show relatively high beneficial results from heated soils. The Solanaceae and Leguminoseae

are on the other hand apparently more susceptible to the toxic action, although great variation exists within the families in this respect. This seems roughly to correspond with the action on seed germination, but experiments to be referred to later along this line apparently fail to bring out any decided correlation.

Length of time of heating. The work of Seaver and Clark (68) and of Pickering (50) have indicated that the length of time the soil is held at a certain temperature does not materially influence its behavior toward plant growth. Some preliminary experiments on this aspect of the problem tended to corroborate their conclusions, but on the other hand there appear to be too many exceptions to permit of applying this conclusion as a general rule. Fine sandy loam soil heated to 115°C. for various lengths of time ranging from 10 to 180 minutes and planting to wheat showed that a gradual increase in yield occurred up to 120 minutes of heating but fell off again slightly on heating to 180 minutes. In this case it should be noted that a soil producing only a

TABLE 19

Influence of length of time of heating virgin sandy loam at 115°C. on growth of tomatoes

TIME OF HEATING	DRY WEIGHT AVERAGE TRIPLICATES
minutes	*gm.*
Check not heated	2.28
10	5.57
20	4.77
40	2.63
80	0.50
160	0.10

slight toxic action on heating and also a plant resistant to this toxic property were used. When tomatoes were grown on muck soil a different result was secured. Heating 10 minutes gave an increase in yield of 132 per cent, 20 minutes an increase of only 53 per cent which on heating 80 minutes was again raised to 157 per cent, but again fell off to 118 per cent on heating 160 minutes (plate 3, fig. 1). This type of behavior both with seed germination and plant growth has, however, been observed too frequently to dismiss as insignificant. The behavior seems to indicate that the balance between the toxic and beneficial action is altered by the length of time of heating, probably due to the altered balance between the production and the volatilization of one or more products from the soil on heating. More definite evidence has been secured, however, to show that the length of time of heating may have a marked action on the behavior toward plant growth. Virgin sandy loam was heated for various lengths of time between 10 and 160 minutes at a temperature of 115°C. and set to tomatoes (plate 3, fig. 2). The toxic action as shown by "chemical" injury first appeared in the soil heated for 160 minutes, and appeared successively in the other soils to and including 20 minutes of heating. At 10 minutes an almost immediate bene-

ficial action was found and persisted throughout the experiment. The soils heated for 20 and 40 minutes recovered in considerable measure and made a better growth than the checks, but they still showed the toxic action on the lower leaves at intervals. The soils heated for 80 and 160 minutes gave practically no growth of tomatoes, the leaves dying off almost as rapidly as formed. The average yield from triplicate pots are shown in table 19. It is at least very evident that the length of time of heating certain soils does influence the growth of certain plants.

Repeated heating of soils. Practical plant culturists using sterilization by heat often inquire as to whether soils once sterilized may again be sterilized and replanted with the assurance of results as good as produced by the first sterilization. Some experimental evidence has been secured on this point in two different ways: first by repeatedly sterilizing a soil after each successive crop, and second, by sterilizing a soil on successive days before planting. The results of the first tests, in which fine sandy loam was used and grown to lettuce, indicated that the soil responded in a beneficial way after each sterilization in very much the same way as previously unsterilized soils did. In another experiment in which wheat was used on muck soil and on virgin sandy loam, and the same soil was heated 1, 2, 4, and 8 times on successive days to 115°C. for 90 minutes, no marked difference in results was obtained, a beneficial action being exhibited in all cases above the unheated check. Some indication, however, that one heating was more beneficial (or less productive of toxic action) than repeated heating, is indicated by the figures (table 20). Another experiment conducted in essentially the same manner, using greenhouse compost grown to tomatoes, again indicated that repeated heating on successive days increased the toxicity to tomatoes, although all the heated soils eventually recovered and produced final yields greater than the unheated checks (table 20). It does not follow, however, that repeated sterilization of the same soil may not be disadvantageous in certain cases and for other reasons not discussed here.

Influence of temperature of soil following sterilization. The results obtained relative to the toxic or beneficial action of heated soils are found to be influenced to a considerable degree by the temperature of the moist soil following sterilization, and this may explain some of the variations which have been secured in different experiments carried on at different seasons of the year. Russell (64) and Koch and Luken (32) have previously noted that results secured on heated soils varied with the time of the year, although they did not apparently connect up this idea with soil temperature in particular. Pickering (50) in studying the loss of the toxic action of heated soils found that its temperature influenced the loss of toxicity to seed germination. It was noted by the writer, in connection with some experiments dealing with the influence of soil temperature on the development of the root-rot disease of tobacco (*Thielavia basicola*), in which steam sterilized soils were used as checks, that retarding action and chemical injury were considerably more marked at soil

temperatures below about 25°C. than at higher temperatures (plate 4, fig. 1). This toxic action was so great as to materially interfere with the progress of the experiments, and it finally became necessary to use special methods of sterilization to avoid it; although the toxic action could be materially reduced by proper methods of handling the soil after heating and before planting. In order to study this action further experiments were conducted with virgin sandy loam soil heated to approximately 100°C. and planted to tomatoes in soils kept at various temperatures together with checks in unheated soils. The tomatoes in unheated soils at soil temperatures below 20°C. made a poor growth and showed increased pigmentation, while those at soil temperatures above 23°C. and up to 32°C. made a good growth (plate 4, fig. 2). In the heated soils the tomatoes at 23–24°C. were practically killed by the toxic agent. The toxic action at lower temperatures was also great but not as

TABLE 20

Effect of repeated heating of soil on successive days to 115°C. on yield of crop grown

SOIL TREATMENT	DRY WEIGHT		
	Muck soil (wheat) average of duplicates	Virgin sandy loam (wheat), average of duplicates	Greenhouse compost (tomatoes), average of triplicates
	gm.	*gm.*	*gm.*
Not heated	4.75	4.85	3.97
Heated once	9.10	7.35	9.73
Heated twice	7.70	6.90	9.00
Heated four times	8.80	5.65	7.50
Heated five times			8.17
Heated eight times	6.55	7.00	

marked as at 23–24°C. At a temperature of 27–29°C. practically no "chemical" injury occurred, although some retardation in growth took place. It is very evident from this and other experiments that the results secured with heated soils are to a great extent influenced by the temperature of the soil. This response is believed to be due largely to differences in bacterial activity as influencing loss of toxicity of the heated soil. The reasons for this explanation will be evidenced by the experiments described later.

Other factors concerned. In addition to the factors already mentioned as being influential in determining growth on heated soils, several others would bear discussion at this time. One of the most important factors, which will be left for later discussion, is the reinfestation of the soil with normal soil flora which has a fundamental influence on the toxic and beneficial properties exhibited. Among the minor factors should be mentioned that of the moisture content of the soil both during heating and during subsequent plant growth. Excessive moisture in the soil during heating as well as during plant growth seemingly predisposes the plant to the injurious action, or increases

the amount of the toxic property in the soil. At any rate watering should be carefully regulated in heated soils, especially in experimental work. This is particularly true for the reason that once a sterilized soil is overwatered, great difficulty may develop in attempts to dry it out rapidly.

The period between the time of heating and the time of planting will influence the amount of toxic and beneficial action exhibited, if the soils are kept under ordinary conditions for plant growth during that time, but not if the soils are kept dry. Whether atmospheric conditions except in so far as they influence soil environment are of any degree of importance in determining the extent of the action of heated soils is not yet known; although they presumably may show some influence as suggested by Russell and Petherbridge (64).

Qualitative changes in plant growth on heated soils

The differences to be found in the response of seeds and plants to heated soils, aside from the more common measurable phenomena, are frequently so marked as to distinguish the heated from the unheated soils. These differences have been generally noted by practically all workers on sterilized soils in the increased vigor and deeper green color of plants grown on sterilized soils. Russell and Petherbridge (64) have gone considerably further in describing these qualitative responses, noting especially the formation of purple pigments in plants grown on sterilized soils as well as changes in the character of the root system.

With respect to seed germination, aside from rate of germination, it may first be noted that many seeds, especially seeds of leguminous plants, apparently become much more easily over-run with fungi when germinated on heated soils. This fact is probably due to the absorption of the favorable property for fungus growth from the heated soil solution. In soils heated to 250°C., especially in those which are most toxic upon heating, the tip of the radicle may often become browned or blackened from contact with the surface of the heated soil. Lettuce seeds on the most toxic heated soils frequently germinate in an abnormal manner, in that the cotyledons emerge from the seed coat before the radicle. In other cases the radicle may make a long spindly growth, seemingly in an attempt to grow away from the toxic medium. The lack of formation of root hairs as compared with the abundant formation on unheated soils is especially noticeable at some stages of germination and growth on heated soils. If the seeds are allowed to continue germination on the surface of heated soils, it may often be noted that the roots instead of penetrating into the soil, grow along the surface of the soil. When roots are, however, forced to grow into certain sterilized soils in order to maintain the life of the plant, the roots may be much retarded in growth and may become short and stubby, without root hairs, frequently discolored as a whole or in local areas, or deeply split radially, partly decayed and sometimes entirely killed. This is especially true in sterilized soils grown to plants under sterile

conditions. It seems reasonable to suppose, therefore, that the retarded early growth of plants on heated soils may in some instances be due in part to the failure of the root system to penetrate the soil with the consequent reduction of their functions, rather than to the absorption of the toxic principles of the plant. This point is illustrated in plate 5, figure 1 showing the retardation of the root system of tomatoes grown at different soil temperatures. That toxic principles are absorbed is shown by the cases of "chemical" injury obtained. That absorption of toxic properties occurs is also shown in seed germination. Certain seeds, of which lettuce may be cited as an example, behave very peculiarly on the most toxic of the heated soils, heated soil extracts, or products of dry distillation of soils as already referred to. This is shown especially by the swelling of the seed to two or three times its normal size, together with a decided change in color of the interior seed coat to a greenish black. The seed in such cases is practically always killed and sometimes bursts the outer seed coat due to internal pressure. The inner seed coat is very tense and hard, and upon pressure, bursts, exuding a droplet of clear liquid. This reaction with lettuce seed is sufficiently characteristic to make it valuable as a qualitative test of toxic agents and will be referred to as such in the consideration of the nature of toxic properties produced in heated soils.

The qualitative responses of growing plants to heated soils are numerous and varied in type. Russell and Petherbridge (64) have made some observations on this, especially with tomatoes. With regard to the formation of a purple pigment the impression is left that the pigmentation is characteristic on heated soils particularly. This does seem to be the case, however. Tomatoes grown on a wide variety of soils and under several different conditions may produce excessive purple pigmentation. Soils low in fertility, or cold soils especially, seemingly respond in a similar manner to heated soils in pigment production. Purple pigmentation on heated soils is not peculiar to tomatoes alone, but seems to be a response more or less common to other plants, being especially noticeable in cabbage and lettuce, as far as observed in these experiments. Although pigmentation sometimes occurs on heated soils in case of tomatoes, it is not necessarily associated with heating. It may occur in the early plant growth, and finally disappear in the later growth; its persistence when present, apparently is in proportion to the unfavorableness of the soil for plant growth.

Another type of color reaction which has been noted in the case of tomatoes, but which may be associated with stimulated as well as retarded growth, is a yellowish green mottling or mosaic appearance of the leaves resembling to some extent the Mosaic disease but usually without malformations (plate 6, fig. A). This abnormality also usually disappears in a short time and is apparently associated with diminished activity of the chlorophyll bodies due to the absorption of a toxic substance fron the soil. In other plants such as tobacco, mottling may not occur but rather a uniform yellowing, especially at the leaf tips or margins.

The most marked effect of heated soil on plant growth, however, is that which has been termed "chemical" injury. This is characterized by distinct spotting of the leaves or lesions on the stem, sometimes the latter occurring at the surface of the soil and resembling the damping-off disease. These spots or lesions often become confluent and the entire leaf or plant may wither and die. In some instances this action has been observed to become localized just below the terminal bud, resulting in the death of the latter and consequently the premature branching of the plant. In other instances "chemical" injury appears in the early stages of growth of the plant with the result that they may be killed outright or remain for weeks or months without making appreciable growth. Where the action has not been too severe, however, the plants may entirely recover and produce greater yield than in unheated soil. On the other hand this type of injury has been observed in plants which apparently made normal growth during the early stages of growth but finally showed symptoms of chemical injury in a late stage of growth (plate 5, fig. 2). The first signs in such cases are sometimes in the veins and midribs of the leaf, and are exhibited by the browning of the veins and curling of the leaves or at other times by spots which become confluent resulting in the entire leaf drying and dropping from the stalk. This condition has all the appearances of a disease caused by a parasitic organism. On account of the fact that it is not always associated with retardation, it appears that it is not necessarily the result of a product formed during heating of the soil but may be the result of a substance developed subsequent to heating. It is, however, also probable that the explanation of this response occurring several weeks after heating the soil may be due to the occurrence of certain environmental conditions favorable for its production or manifestation, as, for instance, modified soil temperature or moisture conditions. This conclusion is supported by the previously mentioned soil temperature experiments and by the observation that overwatering heated soils may increase this type of toxic action.

Similar "chemical" injury which may be mistaken for diseases of parasitic origin have been found to occur upon several other plants grown on heated soils, particularly upon soybeans, cow peas, and tobacco. In the case of soybeans, the browning of the veins of the leaf is especially striking. If this action affects the leaves when they are young and in a rapidly growing condition, it results in leaf curling (plate 6, fig. E). The condition is similar to that sometimes occurring on tomatoes. The spotting of the leaves of soybeans on heated soil usually occurs on the leaf margins and is seemingly associated with the rapid transpiration at these points (plate 6, fig. D). In the case of cow peas, the spotting is far less distinct on the leaves, the spots being much smaller, usually not larger than a pin head, and raised rather than sunken (plate 6, fig. F). In the case of tobacco, the spots resemble very much certain leaf-spots occurring in the field, some of which are of bacterial origin and others probably of a non-parasitic nature (plate 6, fig. C).

These qualitative responses are much more marked in some soils than in others. They may occur in a wide range of soil types and have seemingly no relation to the nitrogen content of the soil. They are influenced by environmental conditions of the soil especially, but they do not appear to be necessarily associated with degree of toxicity as represented by retardation of plant growth in heated soils, in view of the fact that they may appear on plants benefited in their growth by heated soils as well as on plants which are retarded. The relation of soil temperature to this toxic action has been especially demonstrated in the case of tomatoes and tobacco. It was found that "chemical" injury to tomatoes and tobacco on heated soils usually occurred at soil temperatures between 15° and 25°C. and not at higher or lower temperatures. In later experiments, however, it became evident that this injury might occur at lower or higher temperatures if other conditions, such as greatly increased moisture content of the soil, occurred. All observations point toward the fact that a fairly delicate relation exists between the extent of "chemical" injury and the balance between soil moisture and soil temperature. In the acid sand (lime requirement 9.38 tons per acre) the "chemical" injury was marked following sterilization. If neutralized with calcium carbonate before sterilization, this injurious action did not occur. All evidence has gone to show that this toxic action is most marked in acid soils following sterilization.

Other minor qualitative responses of plants to heated soils occur, such as increased tendency toward branching in the case of soybeans. Under greenhouse conditions at least, the soybeans normally drop most of the lateral buds as well as the lower leaves and the plants tend to grow tall and spindly rather than short and bushy. Although this condition may be especially associated with infertile soils, it is seemingly also associated with reduced light. In heated soils, however, these lateral buds do not fall off as readily in most instances but persist and produce short stout leafy branches deep green in color, even in cases of marked retardation due to toxic action.

Some mention is to be found in the literature of increased susceptibility of plants to disease as a result of being grown on heated soil. Wilson (78), for instance, reports wheat rust (*Puccinia graminis*) and wheat mildew (*Erysiphe graminis*) more serious on weakened plants grown on soils heated to high temperatures, than on more vigorous plants grown on soil heated to lower temperatures. The probable changes in susceptibility to disease is to be considered in connection with a separate paper on the use of heated soils in phytopathological research.

The development of fungi in heated soils

That certain fungi grow very well on burned-over areas has probably been noted, with only passing comment, for a very long time. Tacke (74), however, in studying the effect of the heating of soil on the solubility of nitrogen, noted

that fungi grew very well on the extracts of heated soils and believed that this was due to the excellent nitrogen supply present. Kurzwelly (39) reported that various species of fungi not living in soil before sterilization flourish vigorously afterwards. Kosaroff (35) noted, especially, the development of *Pyronema confluens* on heated soils where it did not occur on unheated soils. He concluded that the extraordinary development of this fungus on heated soil was not due to the resistance of the spores of the fungus to the sterilizing action but rather to the destruction of a substance toxic to the development of *Pyronema* in ordinary soils. Seaver and Clark (67, 68) began a series of investigations on the development of *Pyronema omphiloides* on heated soils, in an attempt to explain the reason for this behavior and inclined toward the belief that the beneficial action upon fungi was due to the increased concentration of the food supply.

In the open and especially in the greenhouse in the presence of air-borne spores, the heated soils rapidly became covered with a thick mat of *Pyronema confluens* (Tul.) or *Pyronema omphaloides* (Bull.), Fuckel. Under favorable environmental conditions the soil may on the other hand become overrun with various ordinary molds, especially those of *Penicillium* and *Mucor*. It appears, however, that heated soils are considerably more favorable for *Pyronema* than for *Penicillium* and other fungi, but no more favorable for the latter than for other forms. The common occurrence of their spores in the air, probably explains the presence of certain fungi on the heated soils in many instances. Where other fungi, such as *Rhizoctonia*, *Pythium*, *Thielavia*, *Fusarium*, and other organisms commonly inhabiting soils are reinoculated into heated soils, they apparently find it a very much more favorable medium for growth than unheated soils.

Although no detailed experimental work concerning the relation of fungi to heated soils was carried on in connection with the experiments of the writer, yet considerable observational data are at hand, which are seemingly in some instances contradictory to some of the conclusions of the above named authors. The relation of the following factors in regard to the growth of fungi upon heated soils has been especially noted: temperature to which soil is heated, influence of soil type, influence of soil reaction, length of time of heating, growth on soil extracts, and the effect of soil temperature and moisture on fungus growth in heated soils together with certain correlations between fungus growth and various other factors studied. The observations have shown that the growth of fungi in soils heated to different temperatures is best at about 250°C., the growth either occurring later or being less profuse or entirely absent in soils heated to higher or lower temperatures (plate 7, fig. 1). The range for the development of *Pyronema* appears to be from about 100°C. to 350°C., temperatures above or below this usually giving no growth whatever. This is seemingly true for practically all soils studied, but much more marked in some than in others. It should be noted at once in this connection that there is a close correlation between this growth and various other phe-

nomena studied in soils heated to different temperatures, i.e., toxicity to seed germination, early plant growth, and beneficial action to late plant growth. It may be, therefore, that the property which is favorable for the development of *Pyronema* is the same which is toxic to seed germination and early plant growth as believed by Seaver and Clark (68), but no further evidence of this theory could be found; while on the other hand some contradictory evidence has been secured.

The extent to which fungi, especially *Pyronema*, develops varies greatly for different soils when heated to 250°C., it being on the whole greater on those soils high in organic matter and less in those low in organic matter, although no definite proportion in this respect has been established. Comparing the development of *Pyronema* on the various soils used, it was usually found to be most profuse on the peat and least profuse on the Norfolk sand. It was interesting to note that *Pyronema* in one instance grew most profusely on peat soil heated to 250°C. which was so toxic to tobacco and other plants transplanted into it, that they were killed in a few days. It has been observed, however, that *Pyronema* has made an appreciable growth upon red clay and upon practically pure sand heated to 110°C. The relatively low concentration of the soil solution in these cases raised the question as to the importance of concentration of soil solutions in connection with fungus growth on these soils, and this point will be discussed briefly in a later paragraph. The reaction of the soil within wide ranges (lime requirement of 9.38 tons per acre to one with corresponding alkalinity) seemed to have no influence on the rate of growth of *Pyronema*. In regard to the influence of moisture supply on the development of *Pyronema* on heated soils, it was found that the best development was on soils kept at one-half saturation. Somewhat less growth occurred on soil kept at one-fourth saturation, followed by that at three-fourths saturation with relatively poor growth. No growth was obtained on soil kept at full saturation. No growth, of course, is obtained on soil which is air dry, but it should be especially noted that the property favorable to the development of *Pyronema* may be kept indefinitely in air dry soils, whereas it is relatively rapidly lost in moist or wet soils.

The time of heating the soil has some influence on the growth of *Pyronema*, it being found for instance to occur first on soil heated to 115°C. for 160 minutes, and then appearing gradually on soils heated for 80, 40, 20, and 10 minutes. In this case no correlation seemed to exist with the growth of tomatoes on this soil. It has generally been found that an appreciable increase in concentration of soil solution and ammonia occurs on continued heating of soil at 115°C. The development of *Pyronema* upon the surface of soils maintained at different constant temperatures offers some interesting suggestions. At temperatures above 28°C. no growth of *Pyronema* has been observed. At temperatures ranging between 20° and 28°C., *Pyronema* usually appeared soon after the heated soil had been moistened, fruited, and then disappeared in a relatively short period of time. Finally growth appeared gradually upon

the soil at lower temperatures, and at temperatures of 10° and 15°C., the fungus persisted and grew for several weeks, an occurrence which is relatively uncommon under ordinary conditions. The late appearance and slower growth at the lower temperatures is, no doubt, partly due to a temperature relation effecting the organism itself, but it is also reasonable to suppose that the persistence of *Pyronema* at these lower temperatures is due also to the maintenance of the favorable property in the heated soil, and its early disappearance at the higher temperatures is due to the rapid loss of this favorable property for its growth.

That heated soils are also favorable to the growth of bacterial organisms is evidenced by the fact that extracts of heated soils left exposed to the air or inoculated with bacteria soon become teeming with organisms. Lodge and Smith (41) in experiments with decoctions of sterilized and unsterilized soil found that bacteria may or may not be favored in growth by the sterilized soil decoction, depending upon the nature of the soil. The extract of soil heated to 250°C. has been repeatedly noted in my experiments to be more favorable to the growth of bacterial organisms than extracts of the same soil heated to higher or lower temperatures. This has also been shown by making up soil extracts with 2 per cent agar and inoculating tubed slants with pure cultures of *Bacillus subtilis.* The growth in heated soil extract agars was best in the 250°C. extract and poorer in the higher or lower temperature extracts. The growth of *Penicillium* in sterilized soil extracts from heated soils was approximately in the same proportion and in the following order: 250°C. extract best, 200°, 150°, 100°, 350°, 50°, and unheated poorest. As noted by Seaver and Clark, it is practically impossible to keep soil extracts from being very strongly invaded with lower microörganisms without keeping them under sterile conditions.

The nature of the toxic and beneficial substances produced on heating

The earliest theories in regard to the cause of the injurious action of heated soils were largely founded on the effect of heat on the physical properties of the soil, particularly on the destruction of vegetable matter in burning, although Davy (12) as early as 1819 did not agree with these theories. Arends (1) believed that bad results follow burning due to exhaustion of fertility. Struckmann (73) suggested that certain inorganic substances may be produced which are injurious. Dietrich (13) was first to carefully observe the injurious action of heated soils, and concluded that there was a poison formed from the alteration of the organic matter, and that according to the amount of this poison formed and the sensitiveness of the plant to it, either the injurious or the beneficial action predominated. Where the plants were less susceptible to the poison, there was observed an increased absorption of soluble nitrogen by the plants.

Schulze (66) found a harmful effect of steam sterilization of soil which he attributed to increased solubility of the humus. The effect was found to vary

with different soils and plants, and to be more marked in early stages of plant growth.

Pickering (50) in 1908 published the first of a series of four papers on the action of heated soil on seed germination and plant growth and the changes occurring in the heated soils. His work was especially concerned with the toxic action of the heated soil, although no special attempt was made to determine any definite injurious compound. Extensive studies were made particularly concerning the relation of the increased solubility of organic and inorganic material in the soil due to heating. These papers are of sufficient importance in connection with the investigations reported here to briefly review each separately. Pickering's conclusions were more or less modified in each succeeding paper, however, and it is somewhat difficult to determine to which points most significance should be attached in a brief review.

Pickering's first paper (50) brought out some very interesting conclusions which are further extended in the following papers. The unfavorable effect on seed germination was found to be proportional to the content of organic matter when different heated soils were compared. In general the "richer" and more favorable the soil for germination before heating, the more inhibitory it becomes after heating. He believed that the destruction of the inhibitory substance in heated soil, as a result of added extracts of unheated soil, is due to a process of oxidation. No appreciable destruction of the toxic property resulted when soil was kept for several months at a low temperature in a moderately dry condition. At higher temperatures, however, in presence of moisture, some of the inhibitory properties were lost, probably through oxidation. The inhibitory substance was found not to be of an acid reaction. The retarding effect could not be explained by alteration of bacterial flora in the soil as the alteration extends progressively beyond that necessary to destroy all bacteria. The maximum of toxic effects to seed germination was found to be produced on heating to 200°C., although in a second paper (51) 250°C. was said to be the optimum temperature for toxicity. In this paper Pickering also concludes, contrary to his first paper, that the "poverty" or "richness" of a soil under ordinary conditions bears no relation to its behavior when heated. There appeared to be no connection between the total nitrogen in the soil and the extent to which it was altered by heat, and the inhibitory substance produced was not apparently the same in all cases. Pickering's third paper (52) concludes, relative to the nature of the toxic agent, that the increase in soluble matter produced by heating a soil and the accompanying toxic properties detrimental to the germination of seeds are gradually reduced by exposing the soils in a moist condition to the air, even under aseptic conditions, but are not reduced when the soils are kept moist in the absence of air. The destruction of the toxic substance is therefore thought to be due to oxidation.

In a fourth paper (53) concerning plant growth in heated soils, Pickering concludes that the substances favorable for plant growth and the substances

unfavorable for plant growth in heated soils are of a different nature. The toxic action on plant growth is believed to disappear under the influence of oxidation as it does in the case of seed germination as noted above. Whether or not the toxic agent to seed germination and plant growth is identical cannot be settled but it is provisionally assumed that they are the same. As a result of growing different kinds of plants in heated soils, Pickering concludes grasses are more resistant to the toxic action than non-grasses.

Schreiner and Lathrop (65) heated soils to 135°C. under steam pressure and found an increase in various organic constituents not ordinarily considered in questions of soil fertility. Some of these were beneficial to plant growth and one in particular, dihydroxystearic acid, was found to be harmful to plant growth. These authors are inclined to explain the injurious action produced on sterilized or heated soils to the formation of this toxin and the beneficial action to the formation of other organic compounds. Although the writer attempted to isolate dihydroxystearic acid from the most toxic of the heated soils studied without success, the main objection to this theory in connection with explaining the response of plants to highly heated soils seems to be that it is highly probable that this compound having a low dissociation temperature, would be practically destroyed before a temperature of 250°C. is reached.

The objection to Fletcher's theory (18) that heated soils do not produce toxins, which delay the germination of seeds, but that this delay is caused by decreased imbibition of water from strong solutions of heated soils seems to be contradicted by the observations made in connection with the experiments described in this paper where it was found that certain seeds actually imbibed more water than in normal germination.

Russell and Petherbridge (64) present a considerable amount of data bearing upon the retardation and stimulation of seed germination in soils heated to low temperatures and treated with antiseptics. They made no study, however, of this problem in particular, but were concerned mainly with the beneficial action of sterilized soils upon plant growth. These authors disagree with Pickering as to the chemical nature of the toxic substance and state that they could not find proof that the harmful effect of heated soils on germinating seeds passed off after a time. That ammonia might be the toxic agent produced was suggested, but they could find no analytical data to show that any relation existed between it and the amount of retardation or acceleration to seed germination.

Lyon and Bizzell (42) have also made a careful study of the substances produced in steamed soils injurious to plant growth. They found that the time required for the various soils to recover from this injurious action was, with one exception, in the order of their relative productiveness. Steaming was found to increase the soluble nitrogenous compounds and also phosphoric acid. The nitrates were reduced to nitrites and ammonia, but most of the ammonia was formed from organic matter. They concluded that the toxic

substance produced is a controlling factor in productivity of steamed soils. Apparently, however, they do not suggest anything further as to the chemical nature of the injurious property.

Practically all the results of previous investigations on the nature of the toxic agent in heated soils have been based on results secured on heating below the "critical" temperature. It seemed to the writer, therefore, that a detailed study of soil heated to the optimum for the production of the injurious action might lead to conclusions more satisfactory than those secured from lower temperatures of heating. That the substance toxic to seed germination increases with the rise in temperature up to about 250°C., and that on further heating, the toxic property is gradually lessened until it has entirely disappeared before a temperature of 500°C. is reached, has repeatedly been found to be true. It is reasonable to assume, at least, that at about 250°C. a balance is reached between the maximum production and maximum retention of the toxic substance by the heated soil. Whether the substance is destroyed at temperatures above 250°C. or merely volatilized is not exactly clear. There is some reason to presume, however, that the toxic substance is gradually produced in increasing amounts up to 250°C. and beyond, but that it is rapidly volatilized above 250°C.

Water extracts of heated soils

Water extractions were made of soils heated to various temperatures and the comparative rate of seed germination obtained. The results for water extracts of Waukesha silt loam are shown in table 21, where it may be seen that the toxic property is at least partly soluble in water. Considerable variation is shown in the germination percentages after 24 and 42 hours. This may be due in part to experimental error, but is also seemingly a further indication of similar conditions repeatedly noted in which certain soil heated to 50°C. are more injurious to germination than unheated soil or soils heated to 100°C. This condition has been found to recur too often to be considered merely accidental and yet no explanation can be offered for it at this time as it appears to bear no consistent relation to any of the changes in the soil which have been studied. The germination after 66 hours, however, as shown in table 21 does suggest that the amount of toxin soluble in water is in proportion to that in the soil itself. In some cases the extraction of a soil heated from 200°C. to 300°C. with an equal weight of water yields an extract considerably more toxic than the soil itself. Such marked toxicity is not usually obtained, however, but it is not improbable that the increased injurious action of the extract, beyond that of the soil itself, is due to the separation of the toxin from the ameliorating effect of the absorptive power of the soil.

In order to arrive at some approximation of the proportion of the toxic matter extracted with water in repeated extractions of the same soil, the experi-

ment detailed in table 22 was performed, two different soils being used. The results show that a large proportion of the toxicity was removed at the first extraction and that the second and third extractions with water yielded considerably less of the toxic property. Since the property toxic to seed germi-

TABLE 21

Relative rate of germination of seed on water extracts of soil heated to different temperatures; Waukesha silt loam; lettuce seed

TREATMENT	GERMINATION AFTER					TOTAL
	24 hours	42 hours	66 hours	90 hours	114 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated	30	93	99			99
Heated to 50°C.	3	68	98	99		99
Heated to 100°C.	24	90	99	100		100
Heated to 150°C.	1	7	74	98	99	99
Heated to 200°C.	0	13	50	79	92	92
Heated to 250°C.	0	1	17	76	97	97
Heated to 300°C.	34	93	99	100		100
Heated to 350°C.	2	41	100			100

TABLE 22

The toxicity of repeated water extractions of soil heated to 250°C. on seed germination; lettuce seed

SOIL	TREATMENT	EXTRACTION	GERMINATION AFTER					TOTAL
			42 hours	66 hours	90 hours	114 hours	138 hours	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Waukesha silt loam	Check	First	77	96	97	98		98
	Heated	First	1	2	17	76	78	79
		Second	11	40	59	74		75
		Third	57	80	85	92	93	94
Virgin sandy loam	Check	First	98	99				100
	Heated	First	2	7	26	69	76	77
		Second	9	50	65	87	89	89
		Third	45	82	86	93	94	94

nation is readily soluble in water, further study upon heated soil extracts was made.

That this toxic principle is extractable from all types of heated soils is shown in table 23, although it is not proportional to the toxicity exhibited by the soils themselves. Peat and muck and especially the fine sandy loam, for instance, ordinarily do not exhibit as great a toxicity to seed germination as Waukesha silt loam. The extracts of these first-named soils, after hav-

ing been heated, are, however, considerably more toxic than the heated soils themselves, and slightly more toxic than the extract of Waukesha silt loam. This is especially significant when it is remembered that the concentration of the soil solution used is far less than that actually existing in the soils themselves. The absorptive capacity of the soil, then, has seemingly a most profound influence not only upon the total toxicity exhibited by a soil but also upon its relative toxicity, and this is apparently roughly correlated with the total amount of organic matter in the soil.

TABLE 23

Relative rate of germination of seed in water extracts of different soils heated to 250°C.; lettuce seed

SOIL	GERMINATION AFTER							
	42 hours	66 hours	90 hours	114 hours	138 hours	152 hours	176 hours	200 hours
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Distilled water	60	78	82	87	90			90
Peat	0	0	0	0	0	2	6	9
Muck	0	0	2	5	20	27	35	39
Waukesha silt loam	0	4	20	44	65	72	76	81
Fine sandy loam	1	3	9	26	53	63	69	75
Virgin sandy loam	2	8	32	53	76	80	82	82
Clay	2	10	19	33	61	70	73	78
Norfolk sand	21	51	73	87	89	91	93	

TABLE 24

Seed germination in distillates from extract of heated soil. Waukesha silt loam heated to 250°C.; lettuce seed

FRACTION	GERMINATION AFTER						TOTAL
	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Check (distilled water)	89	99					
First (gaseous)	2	4	14	36	44	45	45+
Second	1	3	13	22	48	63	66
Third	4	11	38	63	84	90	92
Non-volatilized	0	5	31	80	89	92	92

Further evidence upon the nature of the toxic property is obtained by distilling the soil extract. A rough fractionation was made of a water extract of Waukesha silt loam heated to 250°C. The fraction (table 24) which came over before the soil solution came to the boiling point showed almost as much toxicity to seed germination as the second portion and considerably more than the third, which came over after boiling. That all the toxic substance was not volatile is shown by an active toxic principle apparently still remaining in the distillation flask.

Further evidence of the volatility of the toxic principle in heated soil was procured in several different ways. Heating soil in closed vessels usually produced more properties toxic to seed germination than heating in open vessels. Drawing a current of air through the soil into water while heating yielded toxic properties in the water. Drawing air through the dry soil after heating also removed some of the toxic principle.

It has been hoped that the difference in reaction of seeds of different species of plants would give some clue to the nature of the injurious action. Several experiments were therefore planned for the purpose of comparing the response of different seeds to heated soils and to known chemical substances.

The difference in response of seeds of various kinds to the toxic action of heated soil has already been referred to. In an effort to determine if this reaction bore any relation to the genetical relationship of the various groups of plants, a germination test was performed using several different species of the same family in comparison with various species of other families (tables 25 and 26). The results indicate that a considerable degree of correlation exists in this regard. The *Gramineae* are characteristically "resistant" to the toxic action as are also the *Cucurbitaceae*. The *Solanaceae* and *Leguminoseae* are, however, characteristically quite "susceptible" to the injurious action, although not so much so as lettuce seed. The data at hand are not sufficient to satisfactorily establish this rule even for the families mentioned and cannot, of course, be said to apply to other families of plants although there is a suggestion and natural expectation that this may be true. It is interesting to note here that oats and rye showed some degree of stimulation on Waukesha silt loam heated to 250°C., an occurrence which illustrates the striking resistance of these species to the injurious action as compared with lettuce or clover. On the assumption that different seeds respond differently to different chemicals, it seemed reasonable, therefore, that if a considerable degree of correlation could be found between the germination of different seeds on heated soil and their germination in the presence of certain pure chemical substances of known strength, it would be possible to arrive at some conclusion regarding the composition of the injurious product produced on heated soil.

Production of ammonia in heated soils

Of the substances commonly present in soils, which are at the same time toxic in relatively very small amounts and which have further more been frequently found to be toxic to plants in soil (14), ammonia is probably the most common. Various investigators have shown a small initial production of ammonia in soil on sterilization. Hérbert (24) as early as 1889 noted ammonia production in soil on heating to 100°C., while Kelley and McGeorge (30) and the results reported in this paper as well as that of others have shown large increases on heating to higher temperatures. It was logical, therefore, to closely examine the relation of ammonia to the toxic action of the

TABLE 25

Relative rate of germination of seeds of different families of plants on heated soil; Waukesha silt loam soil heated to 250°C.

FAMILY AND SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		45 hours	79 hours	90 hours	114 hours	138 hours	162 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Gramineae								
Rye	None	76	85	87				87
	Heated	81	85	86				86
Wheat	None	57	96	98				98
	Heated	31	91	98				98
Barley	None	70	97	99				99
	Heated	51	95	98				98
Oats	None	8	75	97				98
	Heated	10	88	98				99
Corn	None	8	28	55	89	94	96	97
	Heated	3	15	46	74	89	94	98
Cucurbitaceae								
Cucumber	None	35	82	87	88			88
	Heated	21	74	85	88			89
Squash	None	36	81	91				91
	Heated	4	38	63	71	72	73	81
Pumpkin	None	66	87	96	98			98
	Heated	11	51	82	93	94	95	95
Muskmelon	None	25	51	72	73	76	77	77
	Heated	4	13	34	40	50	66	69
Watermelon	None			7	32	49	69	82
	Heated			2	17	31	56	85
Solanaceae								
Nicotiana rustica	None		81	85	86			86
	Heated		17	60	72	77	81	84
Egg plant	None		16	38	59	66	69	71
	Heated		0	0	1	8	36	72
Pepper	None		1	3	6	12	34	51
	Heated		0	0	0	3	16	55
Tomato	None		3	32	50	57	63	69
	Heated		0	0	7	34	49	64
Datura	None			21	56	69	75	76
	Heated			0	0	14	42	

TABLE 26

Relative rate of germination of seeds of the legume family on heated soil; Waukesha silt loam heated to 250°C.

SEED	SOIL TREATMENT	GERMINATION AFTER							TOTAL
		45 hours	66 hours	90 hours	114 hours	138 hours	162 hours	210 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Red clover	None	41	80	86					86
	Heated	0	2	4	7	11	14	21	53
Alfalfa	None	22	43	51	52				52
	Heated	1	6	11	15	20	25	34	41
Bur clover	None	21	27	31	32				32
	Heated	8	12	13	15	15	16		18
Tapary bean	None	13	77	84	89	91			91
	Heated	0	52	80	89	91			91
Crimson clover	None		10	14	15				15*
	Heated		0	0	1				1*
Goats rue	None		6	10	12	13			13*
	Heated		0	3	6	10	11	12	12*
Robinia	None		3	6	9	17	20	25	25
	Heated		0	2	5	14	19	23	25
Hairy vetch	None		1	9	13	15	16	17	17*
	Heated		1	3	12	16	17		18*
Geuge clover	None		2	11	21	27	29	31	31*
	Heated		0	0	0	1	2	3	3*
Birdsfoot clover	None			8	10	11	12		12*
	Heated			0	2				2*
Astragalus	None			8	19	30	34	40	46*
	Heated			0	0	1	1	2	5*
Peas	None				20	38	77	82	82
	Heated				6	21	58	74	74

* Seeds molded and failure to germinate may be partly due to this, but the seeds were also low in germinating capacity.

heated soil. That this increase is appreciable on heating to 115°C. and very striking on heating to 250°C. is shown by four separate determinations for two different soils in table 27.

Two soils were now taken and heated to temperatures ranging from 50° to 350°C. and the amount of ammonia produced determined. Here it was found

that on the whole there was a gradual increase in ammonia on heating to temperatures up to 250°C. On heating to higher temperatures, the ammonia content was reduced (table 28). The close correlation between the ammonia content on heating to different temperatures, and the toxicity to seed germination is evident from the data presented. The reduced ammonia content on heating to 60°C. has some semblance of an error in determinations, but on the other hand its frequent occurrence in the experiments together with certain

TABLE 27

Increase of ammonia in soils heated to 115°C. and 250°C.

SOIL	TREATMENT	NITROGEN AS AMMONIA IN 100 GM. SOIL					AVERAGE INCREASE
		Exp. 1	Exp. 2	Exp. 3	Exp. 4	Average	
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
Muck	Not heated	4.20	6.93	3.36	6.58	5.28	
	Heated 115°C.	6.72	8.32	8.26		7.77	47
	Heated 250°C.	31.15	31.01	25.06	30.10	29.33	455
Waukesha silt loam	Not heated	1.96	1.68	1.82	2.9	2.09	
	Heated 115°C.	3.50	3.78	4.34		3.87	85
	Heated 250°C.	18.48	13.72	15.40	17.0	16.15	672

TABLE 28

Increase of ammonia in soils heated to different temperatures

SOIL TREATMENT	NITROGEN AS AMMONIA IN 100 GM. SOIL			
	Muck		Waukesha silt loam	
	Single determination	Increase	Average of duplicates	Increase
	mgm.	*per cent*	*mgm.*	*per cent*
Not heated	3.36		1.75	
Heated to 60°C.	1.54	−118	1.40	−25
Heated to 115°C.	8.26	149	4.06	132
Heated to 200°C.	14.42	329	9.52	444
Heated to 250°C.	25.06	646	14.56	732
Heated to 300°C.	5.88	66	7.32	318
Heated to 350°C.	3.92	16	3.36	92

irregularities of seed germination and plant growth on some soils, as already referred to, leads to the conclusion that some correlation may exist even here. This may be illustrated further by results secured on heating soil for various lengths of time (table 29) where not only the ammonia content was lowered on heating 10 and 20 minutes, but also the concentration of the soil solution compared with longer periods of heating, and these changes were accompanied by reduced germination on the soil but increased germination in the soil extracts.

On comparing the amount of ammonia produced in muck and Waukesha silt loam soil on heating them to different temperatures (table 28), it is found that the ammonia content is uniformly higher in the muck soil than in the Waukesha silt loam. Muck soil is, however, never as toxic to seed germination as Waukesha silt loam when heated to the same temperature. That this increase in ammonia occurs in all soils used and is at its maximum at about 250°C. has been frequently shown in these experiments. The increase on heating to 250°C. has usually ranged from 100 to 1,000 per cent for the different soils. No results such as reported by Potter and Snyder (55) i.e., reduced ammonia content on heating peat to 200°C., have been obtained. No great uniformity in results has been secured in toxicity produced on heating the same soil to a certain temperature at different times owing to the variation in the actual amount of heat applied which variation seems unavoidable where automatically regulated ovens can not be used in all cases. The relative results where different soils were heated at the same time in the autoclaves or ovens, have, on the other hand, been remarkably uniform. It should now be stated that the ammonia content of different soils heated to the same temperature seemingly bears no relation to their toxicity to seed germination. This conclusion is illustrated in table 30 showing the results secured on heating different soils to 115°C., although it might be shown better by soils heated to 250°C. The results are somewhat confusing because of the fact that different soils behave differently towards seed germination in their natural or unheated condition. This is especially marked in the case of Sparta sand, and may be due to a toxic agent which is normally present in this soil, and which is more or less common in all soils, since seed germination is apparently always somewhat retarded on unheated soils or their extracts as compared to germination in pure water on filter paper. In any case, heating of the soil to certain temperatures appears usually to reduce the natural retarding influence to seed germination.

TABLE 29

Effect of heating soil at 115°C. for different lengths of time; Waukesha silt loam; lettuce seed

TIME OF HEATING	NITROGEN AS NH_3 IN 100 GM. SOIL	LOWERING OF FREEZING POINT	GERMINATION					
			On soil after			On soil extract after		
			42 hours	66 hours	90 hours	24 hours	48 hours	72 hours
minutes	*mgm.*	*°C.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	3.0	0.007	97	99	100	0	83	99
10	2.1	0.004	54	98	100	4	84	100
20	2.8	0.001	49	93	100	11	83	97
40	3.5	0.015	67	98	99	2	59	100
80	4.1	0.018	69	97	98	3	71	95
160	4.6	0.026	62	93	97	12	78	94
Distilled water						48	96	98

If heated and unheated soils are extracted with water and the ammonia determined in the extracts, it will be found that a relatively high amount of ammonia exists in the heated soil extract as compared with the unheated soil extracts (table 31). This suggests that the absorptive capacity of the soil for ammonia has been reduced by the heating. With weak hydrochloric acid more ammonia can be extracted, but this extract containing more ammonia is less toxic than the water extract. The toxic substance is then apparently

TABLE 30

Effect of heating different soils to 115°C. in autoclave on ammonia content and on rate of seed germination; lettuce seed

SOIL	TREATMENT	NITROGEN AS NH_3 IN 100 GM. SOIL	INCREASE IN AMMONIA	GERMINATION AFTER			TOTAL
				24 hours	42 hours	66 hours	
		mgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Peat	Check	11.7		43	99		99
	Heated	15.0	28.2	0	48	70	75
Muck	Check	4.2		68	99		99
	Heated	7.4	76.2	0	88	98	98
Waukesha silt loam	Check	3.8		3	87	95	96
	Heated	4.8	26.3	0	80	96	98
Virgin sandy loam	Check	3.0		45	100		100
	Heated	4.1	36.6	5	98	99	99
Fine sandy loam	Check	2.4		56	98		98
	Heated	3.4	41.6	2	93	98	98
Sparta sand	Check	1.9		0	54	80	91
	Heated	3.2	68.4	2	72	94	96
Norfolk sand	Check	1.6		16	88	91	92
	Heated	2.2	37.5	0	49	83	95
Red clay	Check	0.5		7	89	97	97
	Heated	0.5	0.0	1	94	100	100

neutralized by the acid, or in some manner rendered less injurious. This experiment suggests at once both the possible relation of free ammonia to the toxicity of heated soils and the relation of the absorptive capacity of the soil for ammonia to the injurious action.

It is fairly clear, nevertheless, from the data presented thus far that ammonia determinations of the soil as such cannot lead to any clear conception of the relation of ammonia to seed germination. This is largely because of the fact that the toxicity of a chemical substance in a soil is in proportion

to the absorptive capacity of that soil for the substance in question. The great absorptive power of soil for ammonia is well known. It is, in fact, recognized in farm practice that the use of ammoniacal fertilizers on soils low in absorptive capacity may be productive of toxic action on plant growth (14). Due consideration must be given this matter, therefore, in relation to heated soils. Several angles of the problem present themselves for consideration. Soils of widely differing absorptive capacities have been used in the experiments. The absorptive capacity of each soil is influenced to a considerable extent by the degree of heat applied. That this is not a simple relation is indicated by the marked alteration of most soils as regards absorptive capacity for water when heated (by moist heat especially) to temperatures of about

TABLE 31

Ammonia in extracts of heated soil and seed germination in distillates from extracts with magnesium oxide; heating to 250°C. and extracted with distilled water and 2 per cent HCl; lettuce seed*

SOIL	TREATMENT	NITROGEN AS NH_3 IN EXTRACT OF 100 GM. SOIL	GERMINATION AFTER					TOTAL
			24 hours	42 hours	66 hours	90 hours	114 hours	
		mgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Waukesha silt loam.........	Check, water extract	0.2	11	88	97	98		98
	Heated, water extract	3.2	0	17	56	60	62	66
	Heated, acid extract	5.1	0	38	74	77	81	87
Virgin sandy loam.........	Check, water extract	0.35	39	97	98	99	100	100
	Heated, water extract	3.7	0	7	16	21	28	38
	Heated, acid extract	5.5	0	16	25	27	41	50
Distilled water....			62	99				100

* The ammonia was determined from a composite of three washings of the soil in equal weights of solvent after standing 24 hours with frequent shaking in each case. The lettuce seed was germinated in a part of about 25 cc. of distillate collected in 5 cc. of water.

80 to 115°C., compared with the very rapid absorption of water in soils heated to high temperatures. This may not be a problem of simple absorption, however, but rather one of capillarity. In general the heating of soil reduces its capacity of absorption, although considerable disagreement may be found in literature in regard to this, due most likely to differences noted in capillarity. If decreased absorption is the rule then in addition to the variation in different soils, it is reasonable to expect that the toxic property produced in a heated soil will vary in one direction in proportion to its natural absorptive capacity. and in the other, in proportion to the degree of heating. The method of solving this problem, however, does not seemingly lie in attempting to obtain correlation between the absorptive capacities of the various soils under different conditions, but in studying the correlation in respect to seed germina-

tion where definite and varying amounts of a toxic agent are added to the soil itself; the main difficulty encountered in this procedure being, however, that it offers no satisfactory direct solution of the problem as between heated and unheated soils.

Two preliminary experiments were made to arrive at a rough approximation of the absorptive capacity of different soils for ammonia, as measured by its toxicity to seed germination, and the response of different seeds to different amounts of ammonia in soils. It will be seen from table 32 that the smallest amounts of ammonia added had no appreciable effect on either the muck or silt loam soil, but that higher amounts stimulated germination and that the highest application retarded germination considerably in Waukesha silt loam. Quartz sand with very low absorptive capacity showed retardation at the lowest percentage of ammonia used. In the same way, where different seeds were used on one soil (table 33), certain seeds showed slight stimulation where the smallest amount of ammonia used was present, whereas others showed retardation. Rye and buckwheat were more resistant to the injurious action of ammonia than clover or lettuce seed. Table 34 illustrates the same facts with a greater variety of seeds. Rye, seemingly the most resistant, was followed in order by wheat, buckwheat, and flax. Garden-cress was the most susceptible (lettuce was not used in this experiment), followed by clover, cabbage, and tomatoes. Beans, cucumbers, tomatoes, and Datura showed an intermediate degree of resistance. If now, the germination of these seeds on Waukesha silt loam heated to 250°C. is compared with the germination on soil to which ammonium hydroxide has been added, considerable similarity will be noted (table 35). With the strength of ammonia used in this experiment, we find that in the case of all seeds, the soils treated with ammonia were more toxic than the heated soils. However, in the case of flax which is very resistant to the toxic action of heated soil, the seeds were practically all killed by the soil to which ammonia had been added. Wheat, buckwheat, and cucumber, which are quite resistant to the toxicity of heated soil, respond proportionally in the same manner to ammonia, and lettuce, garden-cress, clover, and cabbage, which are quite susceptible, respond in a similar way to ammonia. The difference may be noted that in the case of those seeds susceptible to ammonia, practically all the seeds were killed, whereas in the heated soil, the large majority gradually germinated.

The seeming failure of flax seed in this experiment to fall in line with expectations led to a more detailed experiment to determine if this seed would correlate itself in some measure with the other resistant seeds with respect to germination on soil to which ammonia had been added. Garden-cress was chosen as a check seed, as it had shown itself susceptible to ammonia in the previous experiment, and was at the same time quite susceptible to the toxicity of heated soil. Different amounts of ammonia were added to soil both as ammonium hydroxide and as ammonium carbonate. The amount of ammonia in these and in the heated and unheated samples was determined

TABLE 32

Action of different amounts of ammonia added to different soils on seed germination; cabbage seed

SOIL	NH$_3$ ADDED	GERMINATION AFTER 24 hours	32 hours	48 hours	60 hours	78 hours	102 hours	TOTAL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Muck	None	5	38	69	70	73	73	73
	0.02	6	30	64	70	71	73	73
	0.025	14	37	65	70	71	71	71
	0.05	8	32	61	65	68	70	71
	0.1	7	29	65	67	72	72	72
Waukesha silt loam	None	12	28	55	63	64	65	71
	0.02	8	23	68	70	72	74	74
	0.025	6	37	66	71	74	75	75
	0.05	14	40	68	73	76	78	79
	0.1	1	15	47	63	67	71	73
Quartz sand	None	13	32	60	70	73	80	82
	0.02	0	0	19	33	47	62	69
	0.025	0	0	0	0	0	0	0
	0.05	0	0	0	0	0	0	0
	0.1	0	0	0	0	0	0	0

TABLE 33

Action of various amounts of ammonia added to Waukesha silt loam soil on germination of different seeds

SEED	NH$_3$ TO DRY SOIL	GERMINATION AFTER 24 hours	32 hours	46 hours	70 hours	94 hours	118 hours	142 hours	166 hours	TOTAL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lettuce	None	74	85	90						90
	0.04	78	89	96						96
	0.1	3	44	79	82	86				86
	0.2	0	0	0	0	0	0	0	0	0
Clover	None	15	69	88	89					89
	0.04	13	56	83	89	90				90
	0.1	0	0	0	0	3				
	0.2	0	0	0	0	0	0	0	0	
Rye	None	57	86	96	97					97
	0.04	58	90	95						95
	0.1	16	70	91	92					92
	0.2	0	0	0	0	0	0	0	0	
Buckwheat	None	0	0	12	35	56	63	67	68	
	0.04	0	0	9	38	50	61	64	64	
	0.1	0	0	13	43	52	63	65	67	
	0.2	0	0	0	0	0	0	0	0	

TABLE 34

Relative rate of germination of different seeds on soil treated with ammonia; Waukesha silt loam treated with 0.05 gm. NH_3 (25 cc. of 0.2 per cent NH_3 solution) to 50 gm. of soil

SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	162 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Wheat	None	55	97	98				98
	NH_3	51	97	98				98
Rye	None	66	85	88				88
	NH_3	70	81	86				87
Garden cress	None	21	38	53	62	63	65	65
	NH_3	0	3	9	13	17	27	50
Bean	None	43	88	91				92
	NH_3	23	94	97				97
Clover	None	63	82	86				
	NH_3	5	35	45	55	58		60
Flax	None	98	99					99
	NH_3	74	85	88				89
Cabbage	None	47	62	66	67	68		69
	NH_3	12	38	49	52	58		66
Cucumber	None	41	84	85				85
	NH_3	8	76	82	84			85
Buckwheat	None		8	25	36	54	60	63
	NH_3		4	21	28	40	50	61
Tomato	None		18	44	55	58	61	66
	NH_3		2	28	53	58	61	69
Datura	None			43	65	68	69	83
	NH_3			34	55	57	60	97

by the ordinary method in order to aid further in the correlation of facts (table 36). From the data it may be seen that at the end of 54 hours and thereafter, garden-cress is considerably more susceptible to heated soil than flax seed. Where 62 mgm. of ammonia were present, the cress seed was very strikingly retarded and flax seed only slightly. A similar proportion apparently exists where other strengths of ammonia were used. It is believed, therefore, that the results obtained warrant the tentative conclusion that as far as the response of different seeds to the toxic action of ammonia is concerned, a high degree of correlation exists between it and the toxic action on these seeds produced by heating the soil.

TABLE 35

Relative rate of germination of different seeds on soil treated with ammonia and heated to 250°C. Waukesha silt loam 0.075 gm. NH_3 (25 cc. of 0.3 per cent NH_3 solution) to 50 gm. soil*

SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	162 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lettuce	None	53	76	80	81			81
	Heated	0	1	10	18	23	39	58
	Ammonia	1	2	4	4	4	5	7
Flax	None	95						95
	Heated	91	96	97				97
	Ammonia	0	1					1
Wheat	None	53	93	96				96
	Heated	49	91	96				96
	Ammonia	25	73	87	88	90		90
Cabbage	None	4	28	36	42	45	46	46
	Heated	6	3	6	8	18	27	44
	Ammonia	0	0	0	1	1	1	1
Buckwheat	None	53	93	94	95			95
	Heated	50	90	93	94			94
	Ammonia	40	88	90	91			92
Garden cress	None	27	65	71	75	76		76
	Heated	12	26	29	30	38	53	65
	Ammonia	0	0	0	0	0	0	0
Clover	None	47	78	85				85
	Heated	3	21	42	50	61	71	80
	Ammonia	0	3	5				5
Tomato	None	0	22	86	91	92	93	93
	Heated	0	0	21	71	86	88	92
	Ammonia	0	0	38	64	71	73	81
Cucumber	None	0	61	84	86	87		87
	Heated	0	55	82	87	87	89	89
	Ammonia	0	31	62	70	72	76	79

* Contained 14.7 mgm. NH_3 per 100 gm. soil.

Referring further to table 36, however, it may be seen that in the case of both cress and flax greater toxicity to seed germination existed on heated soil with only 20 mgm. of ammonia present than was true when 32 mgm. of ammonia which had been added to the soil directly were present. The supposition that the difference here is due to the reduced absorptive capacity

of the heated soil for ammonia seems reasonable. An attempt was made to test this theory by adding ammonia to soil heated to 250°C. and 350°C. as compared with equal amounts added to unheated soil. For some reason the addition of ammonia to soil heated to 250°C. did not increase the toxicity up to the expectations; the absorbing power for ammonia was apparently still large. On the soil heated to 350°C. however, the absorbing power was greatly reduced as indicated by increased toxicity. Unfortunately no further attempt was made to check up this apparent discrepancy and it must be as-

TABLE 36

Germination of seed on Waukesha silt loam soil treated with ammonia as ammonium hydroxide and ammonium carbonate as compared with heated soil

SEED	SOIL TREATMENT	AMMONIA AS NITROGEN IN 100 GM. SOIL*	GERMINATION AFTER				TOTAL
			30 hours	54 hours	78 hours	102 hours	
		mgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Garden cress....	None	3.8	13	55	85	86	88
	Heated to 115°C.	3.8	11	46	67	72	84
	Heated to 250°C.	20.4	0	8	38	41	79
	NH_4OH 0.1 per cent†	32.0	2	16	58	70	71
	NH_4OH 0.2 per cent	62.2	0	1	6	9	53
	NH_4OH 0.3 per cent	91.0	0	0	0	0	0
	NH_4HCO_3 0.1 per cent	44.0	1	10	46	69	88
	NH_4HCO_3 0.2 per cent	87.8	0	0	0	0	0
	NH_4HCO_3 0.3 per cent	122.4	0	0	0	0	0
Flax............	None	3.8	77	96			97
	Heated to 115°C.	3.8	61	94	97	98	98
	Heated to 250°C.	20.4	6	90	96		97
	NH_4OH 0.1 per cent†	32.0	72	94	95		96
	NH_4OH 0.2 per cent	62.0	42	79	88	91	92
	NH_4OH 0.3 per cent	91.0	0	4	7	8	8
	NH_4HCO_3 0.1 per cent	44.0	35	93	96		96
	NH_4HCO_3 0.2 per cent	87.8	0	7	12	13	13
	NH_4HCO_3 0.3 per cent	122.4	0	0	0	0	0

* Represents amount recoverable by magnesium oxide method.

† Calculated per cent solutions applied.

sumed that if ammonia is responsible for toxicity in the heated soil, that various chemical (61) as well as physical properties of the soil influence its manifestation.

That ammonia exists in the heated soil as an absorbed gas seems improbable. Under such conditions it would be gradually lost through areation even in dry soil. The toxic property is exceedingly stable, however, in perfectly dry soil. The readiness with which it becomes active and is lost in moist soils indicates a very unstable condition in the presence of moisture and

suggests the similarity of the product to such an unstable salt as ammonium carbonate as shown in table 36. For this reason ammonia was added to the soil in proportionate amounts both as carbonate and hydroxide. The result was practically the same as shown by retarded seed germination. It may be assumed that the loss of toxicity in heated soils is purely a chemical question and is due to the breaking down of ammonium salts and the loss of the toxic radical as in the case of ammonium carbonate. But assuming further that ammonia is actually the toxic agent, a difficulty is at once encountered. If ordinary sterilized soils are either naturally or artificially inoculated with normal soil flora, the ammonia content as determined by the ordinary methods shows a gradual increase, together with a reduced toxicity to seed germination. The increased ammonification in sterilized soils has been repeatedly shown by various investigators and has been previously referred to here. The results presented in table 37 show that a reduced toxicity to seed germination has occurred in the face of increased ammonia content during a period

TABLE 37

Germination of lettuce seed on virgin sandy loam after increase in ammonia due to sterilization and re-inoculation

TREATMENT	NITROGEN AS NH_3 IN 100 GM. SOIL	GERMINATION AFTER		TOTAL
		26 hours	42 hours	
	mgm.	*per cent*	*per cent*	*per cent*
Not heated; moist 14 days	4.7	92	99	99
Heated to 115°C.; moist 14 days	11.9	75	97	99
Not heated (kept dry)	2.8	88	99	99
Heated to 115°C.* (just before test)	3.7	11	94	97

* Dry.

of 14 days of exposure to the air. The results indicate a reduced toxicity due to, or in close conjunction with bacterial activity favoring ammonification, and experiments were therefore later undertaken to determine in how far ammonification, or bacterial activity as such, reduced the toxic action of heated soils.

A theory assuming ammonia to be the toxic agent produced in heated soils would appear hopeless here were it not for other evidence. In order to maintain the theory at this point, various phenomena occurring in the fixation of ammonia in soil may be resorted to, not only to explain reduced toxicity due to changed form, but also to show that these forms of ammonia are easily reversible. Assuming that ammonia is the toxic agent, it is possible that it may be fixed or changed, chemically, biologically, or physically (43) into less toxic but unstable compounds which are again readily broken down to ammonia by the relatively violent method of determination of ammonia by distillation with magnesium oxide as used in these experiments. On the other hand,

this method may be quite satisfactory for actual ammonia determinations in heated soils where such decomposition has already occurred, and is perhaps not advanced much further by the distillation process.

Even though a very satisfactory correlation could be obtained between seed germination on soil to which ammonia is added and on heated soil, this would not be sufficient ground to prove the relation of ammonia to the toxic action of heated soils. The objection can be made that many seeds may react in a similar manner to two or more toxic chemical agents. This subject has been considered only in a preliminary way and as far as could be found, the literature upon this point is neither extensive nor very helpful. In a preliminary experiment testing various radically different chemicals such as acids, the strong alkalies, various organic bases, and formaldehyde on seed germination, it was found that the qualitative differences in reaction were sufficiently great in most instances to warrant the conclusion that certain chemicals gave fairly distinctive reactions with certain seeds. The qualitative response of seeds to ammonia has been previously mentioned. The swollen, blackened condition of certain seeds, especially lettuce, exposed to weak strengths of ammonia was not in any case approached by exposure to other chemicals used. That ammonia causes increased imbibition of water by seeds due probably to its action upon the permeability of the seed coat, has been described especially by Brown (7) and it is believed that the swelling of seeds treated with ammonia in these experiments is due to the same cause.

In order to get more evidence upon the similarity of the action of heated soil, its extracts or distillates, and of ammonia upon seeds, histological studies were undertaken. Lettuce seeds which were swollen and killed by heated soil extracts and ammonia were fixed with mercuric chloride, imbedded in paraffin, sectioned and stained, and compared with sections of normal seeds. In both instances the inner seed coat was found to be greatly distended from the cotyledons, which were apparently not greatly affected in size. The color change in the seed was seemingly due to the deposition of a new substance between the cotyledons and the inner seed coat. This substance in sections was of a greenish purple color and seemed to be made up of bodies of a fairly definite structure, and usually more or less globular in form. Although the study of these sections was not carried out in any great detail, the similarity of these bodies to those described by Darwin (11) in roots injured by ammonia was especially noticeable and interesting.

That other substances besides ammonia may react in a similar manner with lettuce seed is not questioned but as yet no chemical substance which is likely to be present in heated soil, seems to produce this reaction. The results on rate of seed germination with formaldehyde as compared with ammonia are given in table 38. This may serve to illustrate the point that different chemical substances may be expected to behave differently in their effect upon the rate of seed germination also. The data indicate that ammonia at the strength used was relatively more toxic than the heated soil in the

case of the four seeds treated. On the other hand, with three seeds formaldehyde was more toxic than ammonia, while in the other case, i.e., the cucumber seed, it was relatively less toxic than ammonia. This evidence seems to illustrate the point that seeds do not on the whole, react in a similar manner to toxic chemical agents.

The distinctive odor of heated soils, especially of the products of dry distillation, point strongly towards certain organic bases such as pyridine, piperedine, or quinoline as being active agents in toxicity to seed

TABLE 38

Comparison of the relative rate of germination of different seeds on sterilized soil and soil treated with ammonia and formaldehyde; Waukesha silt loam heated to 115°C.; treated with 0.3 per cent NH_3 and 1–200 formalin

SEED	SOIL TREATMENT	GERMINATION AFTER 42 hours	66 hours	90 hours	114 hours	138 hours	162 hours	TOTAL
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lettuce	None	83	92	94				95
	Heated	8	40	67	83			84
	Ammonia	10	19	22	23	30		30
	Formaldehyde	0	1	11	33	45	47	47
Flax	None	97	98					98
	Heated	92	94	95				95
	Ammonia	2	6	9	11	12	17	17
	Formaldehyde	0	0	0	0	0		0
Wheat	None	30	93	97				97
	Heated	47	96	97				97
	Ammonia	18	93	98	99			99
	Formaldehyde	8	69	95	98			98
Cucumber	None	5	65	82	84	85	86	86
	Heated	10	71	86	87	88		88
	Ammonia	0	31	76	83	84	86	86
	Formaldehyde	0	53	81	86	87	91	91

germination or plant growth. In the absence of any satisfactory qualitative or quantitative method of determination of these bases in the presence of ammonia, considerable significance can be attached to the qualitative effect of ammonia upon lettuce seed as a means of determination. Pyridine, piperidine, or quinoline, and other related compounds do not cause the increase in pigmentation of lettuce seed characteristic of ammonia, although some may produce swelling of the seed. The response of lettuce seed to ammonia and to highly toxic products of heated soil are, however, apparently identical.

Relation of soil flora to reduced toxicity

The most extensive work upon the nature and loss of toxicity of heated soils has been that of Pickering (50—53). His conception of the toxic property produced in heated soil as concluded in practically all of his papers, is that it is organic and nitrogenous in nature and is destroyed by oxidation. Some reasons for believing that the disappearance of the toxic principle is not due to a simple chemical oxidation process, but that it is due to the activity of soil microörganisms have already been mentioned. In order to obtain further data upon this subject an experiment was performed in which soil was stored for a period of about two months under various conditions as far as aeration, moisture, and activity of microörganisms were concerned. The soil was stored in wide-mouthed bottles of 3 liters capacity and placed in a horizontal position in order to expose as much of the soil surface as possible to the air. These bottles were carefully plugged with cotton. Where it was desired to exclude air, bottles of only sufficient size to hold the soil were used and these were tightly corked and sealed with paraffin. Virgin sandy loam soil was heated to 200°C., thoroughly mixed, and the equivalent of 600 gm. of air-dried soil added to each of 13 containers. In the unheated checks 600 gm. of air-dried soil was used. The soils to be moistened were then watered with an equal quantity of water, plugged as desired and the heated series sterilized in the autoclave for about $1\frac{1}{2}$ hours at 15 pounds pressure. Certain of the soils were then inoculated with various microörganisms while others were kept sterile. The treatment of each soil, together with the results, are shown in table 39. Although the tests were run in triplicates in some instances they are not included in the table. The data on the amount of ammonia present and the toxicity to lettuce seed were taken after 55 days of storage. Transfers of soil were made to determine the nature of the flora present or of possible contamination. The results are believed to be very significant in indicating the nature of the loss of the toxicity in particular. It will be noted that the initial production of ammonia on heating to 200°C., as shown in the unaltered soils, ranges around 13 mgm., three to four times the amount in the normal unheated soil. In the presence of microörganisms and moisture, the ammonia content has appreciably increased, due to the activity of either bacteria or fungi, either in the presence or absence of good aeration. The importance of various fungi as ammonifiers in the soil has been well reviewed and described by McLean and Wilson (44). As was to be expected, the microörganisms had no influence on the dry soil. In the sterile moist soil whether in the presence or absence of good aeration, no appreciable change in ammonia occurred. Comparing the toxicity to seed germination with the ammonia determinations it may be seen that the reduced toxicity is approximately directly proportional to the increased content of ammonia. In other words, the toxicity has been reduced by the microörganisms where present and active as measured by increased ammonification, but has not been reduced appre-

ciably in the absence of these organisms, and in the presence of otherwise identical conditions. Such a biological explanation of the disappearance of toxic compounds from the soil has been recently suggested by Robbins (59). In no case, during the time of storage given and at the temperature and other environmental conditions afforded, was the toxicity completely reduced. It should be remembered, however, that this heated soil represents a much higher degree of toxicity than is ordinarily secured in soil sterilization. The conditions for free access of air were of course not the best even though a cotton-plugged wide-mouthed bottle was used with the soil layer hardly

TABLE 39

Influence of various manners of storage of heated soil on ammonia content and rate of seed germination; virgin sandy loam heated to 200°C.; stored 55 days; lettuce seed

CULTURE NUMBER	TREATMENT						NITROGEN AS AMMONIA IN 100 GM. SOIL	GERMINATION AFTER							TOTAL
	Heated	Sterilized	Inoculated with	Aeration	Moisture	Sterile cultures		27 hours	39 hours	55 hours	76 hours	118 hours	160 hours	232 hours	
							mm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	+	+		+	+	+	13.9	0	0	2	26	52	80	82	
3	+	+	5 cc. soil extract	+	+		20.9	13	68	94	97	97	99	99	99
5	+	+	Aspergillus	+	+		15.8	0	2	10	45	62	88	96	96
6	+	+	Pyronema	+	+		19.5	0	5	40	80	88	94	94	94
9	+	+		–	+	+	13.3	0	0	1	13	26	57	80	96
11	+	+	5 cc. soil extract	–	+		19.8	0	5	11	25	39	79	81	95
7	+	+		+	–	+	13.2	0	0	3	12	23	48	88	
8	+	+	1 gm. dry soil	+	–		13.2	0	0	1	12	41	78	85	94
12	+	+	1 gm. dry soil	–	–		12.6	0	0	1	6	31	65	78	88
13	+	+		–	–	+	13.6	0	0	2	7	35	54	79	92
14	–	–		+	–		3.5	89	98	99	100				100
17	–	–		+	+		4.3	95	97	99	99				99
15	–	–		–	+		7.6	90	97	98	98				98
16	–	–		–	–		3.6	84	93	95	97				97

more than an inch in thickness at its maximum depth. This does not influence the result, however, in showing the comparative importance of bacterial and "mold" activity and aeration alone in reducing toxicity. The least loss of toxicity was obtained in the soil kept sterile, aerated, and dry and the greatest loss in the soil inoculated with normal soil flora in the presence of aeration and moisture. The efficiency of *Pyronema* in reducing the toxicity is especially interesting, and indicates that this fungus so commonly occurring on heated soils may serve a very useful purpose in reducing their toxicity to higher plants.

It may be argued again from this experiment that the increase in ammonia content together with decreased toxicity is evidence against ammonia having

anything to do with toxicity to seed germination. We may assume, however, that ammonia in the presence of microörganisms is fixed or exists in various unstable stages of fixation which are no longer toxic but which may again be reduced to ammonia on boiling with magnesium oxide, and is hence recorded as ammonia present in the soil, though it no longer plays a part in influencing seed germination. That various transition products of ammonia are changed to ammonia by sterilization has been found by Nikitinsky (45) and others.

It is evident that the toxic substance produced in heated soils is soluble in water. Therefore assuming that ammonia is the toxic agent, more ammonia should be found to be soluble in water where its soil content is highest. This was found not to be the case, only about 3.3 mgm. being extractable in the inoculated aerated moist soil, whereas 4.35 mgm. were found in the sterile aerated wet soil. In the inoculated not-aerated moist soil 5.35 mgm. were found to be present, this higher amount being due apparently either to reduced loss of ammonia from lack of aeration or to increased denitrification.

The differences in odor of the soils kept under these various conditions was very marked. Where no activity of microörganisms occurred in the heated soils the odor was the same as that of the heated soils immediately after heating, whether kept dry or moist, aerated or not-aerated. Where microörganisms had been active in the aerated cultures all the odor of heated soils had apparently disappeared, the only detectable odor being similar to that of ordinary soil, except in the case of the Pyronema and Aspergillus cultures where a "moldy" odor was very evident. In the case of the not-aerated moist inoculated cultures, however, a very marked disagreeable odor, similar to that of fermenting manure was present, considerable hydrogen sulfide probably being present. The action of denitrifiers in the absence of aeration no doubt account for this condition. The results in table 39 together with other evidences obtained are believed sufficient to justify the conclusion that the loss of the toxic substance from heated soils is not a simple matter of oxidation proceeding even under aseptic conditions as concluded by Pickering, but is due rather to the activity of certain bacteria and fungi which become reintroduced into sterilized soils. That *Pyronema*, which is commonly found on sterilized soils is very efficient in this respect has been shown and indicates that its preference for heated soils over the unheated soils may be one of relation to ammoniacal nitrogen, just as other fungi, bacteria or green plants differ in forms of nitrogen preferred.

In studying the properties of the toxic or beneficial agent in the heated soils, it is evident that many advantages can be secured by separating the toxic property as much as possible from the body of the soil itself. This may be accomplished by extractions either with water or other solvents, or by means of collecting products driven off from the soil upon heating to different temperatures. This latter method is essentially dry distillation. It is believed that much important evidence secured in the future upon the subject of sterilized soils will be obtained through a more careful study of the

extracts or dry distillation products of heated soils. Aside from the work of Seaver and Clark (68), very little work has been done upon this phase of the problem.

When soil is heated to various temperatures and extracted with water, it becomes evident at once that the color of the soil extract increases gradually with the temperature of heating up to 250°C., while further heating decreases color until practically a colorless extract is obtained from soils heated to 350°C. This has been found to be true for all the soils used, the color varying from a very light tinge of straw color up to a very dark brown wine colored liquid at 250°C. The depth of color varies very much for the different soils, and is roughly in proportion to their content of organic matter. Peat soil extract usually gave the deepest color, whereas extracts of red clay or Norfolk sand showed very little color. The color is a fairly good indication of the toxicity of the extract to seed germination, though germination does not necessarily always decrease with increase in color, i.e., a slightly colored solution may favor seed germination, as for instance, that from certain soils heated to 100°C.

As has already been stated, the fungus development in extracts of heated soil usually increases with the temperature up to 250°C., falling off again at higher temperatures, and the extent of fungus growth can to some extent be correlated with the color of the soil extract as can toxicity to the growth of green plants. These colored solutions are readily decolorized by filtering through boneblack or by other decolorizing agents without apparently affecting their chemical composition. The color is also rapidly lost in the soil, due either to reabsorption or destruction, but may be kept quite indefinitely as bottled extracts, although some changes appear to occur.

Closely associated with color in soil is the odor which is generally quite closely correlated with the color. The odor varies considerably from different soils but is usually quite characteristic, ordinarily being rather pleasant but difficult at times to compare with known odors. Seaver and Clark (68) likened this odor to that of caramel and again to pyridine. The odor of pyridine has also been very characteristic in my extracts together with those of picoline and quinoline. These odors are, however, far less marked in the ordinary extracts than in the products of dry distillation.

Soil extracts toxic to seed germination, if stored in stoppered bottles, can be apparently indefinitely kept without loss of the toxic quality, although some change occurs, especially where no attempt is made to keep the extracts sterile. Even in sterile extracts, however, some precipitation of material seems to occur from the filtered solutions on standing, it being considerably greater with some soils than with others.

Dry distillation of soil

To get a more concentrated form of the toxic property than that which could be obtained by extraction with water, dry distillation was resorted to.

A large bomb holding about 16 pounds of Waukesha silt loam soil was made in such a way that it could be placed in a gas oven to be heated and at the same time permitting air to be drawn through the soil and passed through bottles containing various solutions. The air was drawn through the soil and solutions by means of a filter pump. When drawn into water, the products were found to be toxic to seed germination and slightly acid in character. When evaporated to dryness, however, it became evident that the toxic property as well as the acidity was lost, indicating their volatile nature. Drawing the distillate from heating soil through barium hydroxide or calcium hydroxide showed that enormous quantities of carbon dioxide were driven off. This suggested that the increased acidity in heated soils might be due to the carbonic acid formed, which is partly corroborated by the loss of the acidity of the distillate on boiling. On longer heating and at higher temperatures, however, the distillate in water becomes colored and strongly alkaline, and also takes on the strong pungent odors of picoline, pyridine, or piperidine, masking seemingly some traces of odor of ammonia. Qualitative tests for ammonia with Nessler's showed strong reaction. Two dry distillations were now run with Waukesha silt loam by heating it between 250° and 300°C., and drawing the distillate through a weak solution of hydrochloric acid for several hours. This distillate was then evaporated to dryness and a dark colored salt was secured. It was again taken up with water, decolorized with animal charcoal and finally clean white salt was secured, which in one case amounted to 3.15 gm. and in the other 3.92 gm. from 16 pounds of soil. This was shown to be ammonium chloride by quantitative determination in comparison with chemically pure ammonium chloride. The yield of ammonia in the extracted salt was very close to that of the known salt. Tests of seed germination in the soil after distillation showed it extremely toxic, indicating that only a portion of the toxic agent had been withdrawn. The distillate in water from long dry distillation at 200° to 300°C. is darker in color than that obtained from water extract of heated soils, and has some oily properties which, however, apparently disappear on standing. Its toxicity to seed germination is very great; most seeds are killed when sown on filter paper moistened with the extract. The distillate has been kept in bottles for two years without apparently losing much of its toxicity. Fungi do not grow in the most toxic of these.

If this distillate is redistilled and roughly fractionated, we find that a gaseous product is at first rapidily given off at a rather low temperature. This gaseous substance is very alkaline and very toxic, and qualitative tests show strong reactions for ammonia. The later fractions show less alkalinity, less ammonia, and less toxicity. Not all the alkalinity ammonia, or toxicity, however, can be removed by boiling, indicating that some stable compounds of ammonia or other toxic agents are present. It is also interesting to note that, although the first distillates are colorless, they finally become colored, as a probable result of other substances going over at low temperatures in

addition to ammonia. The characteristic odors also distill over, and this fact, of course, argues against drawing any satisfactory conclusion regarding the relation of the ammonia present to seed germination. In relation to the toxicity of these distillates to seed germination, it should be noted here again that the same distinct qualitative reaction occurs as previously described, i.e., the seeds placed in contact with it are first discolored, in the case of lettuce becoming greenish black, and secondly that the seeds, especially lettuce and clover, rapidly swell to often two or three times their original size, frequently bursting the seed coat. Similar but much less marked results were secured by drawing air through soil heated in the autoclave at 115°C.

With reference to the effect of ammonia upon seeds as observed in this connection, it may be well to mention briefly the observations of others of a similar nature. Pickering (51) also noted that on one heated soil (Takoma loam) seeds were turned black when placed upon it. Bokorny (4) especially has noted the marked toxicity of very small amounts of ammonia upon seeds, he having found usually that 0.01 per cent was very harmful. In a later paper this author makes a special point of the fact that the injurious nature of ammonia is due largely to the ease with which it is able to combine with the cell protoplasm. Brown (7) in studying the selective permeability of the covers of the seeds of *Hordeum vulgare* found that the velocity with which water is absorbed from solutions of ammonia is remarkable. Similar osmotic relations on the part of ammonia have been previously noted by others (49). It is believed that the reaction observed by Brown is similar to the one with which we have been dealing and explains the swelling of seeds on distillates from heated soils. It should be said in passing that the views presented in this respect have been partially suggested by Armstrong (2) in some work upon stimulation of plant growth. In working with ammonia, he concluded that it seems to be the most important active "natural" stimulant, and that carbon dioxide is also effective as a hormone. He finds ammonia stimulates plant growth in small amounts and that it is toxic in larger amounts. Referring to Russell's work (64) and that of his collaborators, Armstrong adds that two powerful hormones are present in sterilized soils, i.e., ammonia and carbon dioxide, the latter being produced by increased oxidation. He believes that increased growth can be ascribed in large part to these, and that retarded growth may be explained by the assumption that they are in excess and that the balance is probably a delicate one.

The Concentration of the soil solution

The importance of the concentration of the soil solution in heated soils as subscribed to especially by Seaver and Clark (68) and Fletcher (18) is such that it seemed advisable to obtain further data upon this subject in relation to the observed conditions in the experiments carried on by the writer. The works of Pickering (51), Seaver and Clark (68), Wilson (79), and Boyoucos

(6) and Koch (33) have all established the fact that increase in concentration of the soil solution occurs on heating soils. Their determinations, however, were not for the most part carried on with soils heated to sufficiently high temperatures to arrive at a maximum concentration, as found on heating to 250°C. The method used in the determinations reported in this paper have been simply that of determining the lowering of the freezing point in soil extracts by means of a Beckmann thermometer. Boyoucos's paper (6) describing a method of immersing the thermometer bulb directly into the soil came after my determinations on soil extracts had been made. The results with soil extracts, however, seem to be equally satisfactory where only comparative results are desired. The method of using soil extracts seemed to

TABLE 40

Lowering of the freezing point of extracts of different soils heated to different temperatures

SOIL	TREATMENT	DEPRESSION OF FREEZING POINT AVERAGE OF DUPLICATE DETERMINATIONS				AVERAGE
		1	2	3	4	
		°C.	°C.	°C.	°C.	°C.
Muck	Not heated	0.019	0.012	0.023	0.026	0.020
	Heated to 115°C.	0.031	0.035	0.033		0.033
	Heated to 250°C.	0.145	0.189	0.086	0.199	0.155
Waukesha silt loam	Not heated	0.009	0.013	0.020	0.021	0.016
	Heated to 115°C.	0.016	0.024	0.040	0.026	0.026
	Heated to 250°C.	0.032	0.056	0.073	0.079	0.060
Fine sandy loam	Not heated	0.027	0.013	0.011	0.012	0.016
	Heated to 115°C.	0.034	0.009	0.014	0.030	0.022
	Heated to 250°C.	0.074	0.044	0.047	0.052	0.054

possess one difficulty, however, namely that although the readings for any one series of solutions gave uniform and constant results, they usually did not compare well with readings made of other extracts from the same soil. Since this variation occurred in the unheated as well as in the heated soil, it is apparently not only due to differences in temperature of heating or time of heating but also to variations in degree of solution. Since certain soils filter with much more difficulty than others, especially those unheated or heated to a low temperature, force filtering was used in such cases, and it was thought that this or a small difference in time of extraction might explain the variation in results. This was shown by preliminary experiments, however, apparently not to be the case. The averaged results are, nevertheless, satisfactory for showing the increase in concentration of the soil solutions in different soils when heated to different temperatures. That such increased concentration is quite marked is shown by the results summarized in table 40. From this table as well as from table 41, it may be seen that the concentration of the

soil extract from muck soil heated to 250°C. is two or three times as great as that of Waukesha silt loam heated to the same temperature. This is not in proportion to the relative toxicity to seed germination or to plant growth and indicates that concentration apparently has little to do with the retarded germination as contended by Fletcher (18) or to toxic action on green plants as proposed by Seaver and Clark (68). Reference to table 41 will show a gradual increase in lowering of the freezing point in the two soils when heated

TABLE 41

Lowering of the freezing point of extracts of soils heated to various temperatures

TREATMENT	DEPRESSION OF FREEZING POINT		
	Muck soil	Waukesha silt loam I	Waukesha silt loam II
	°C.	°C.	°C.
Not heated	0.012	0.021	0.009
Heated to 50°C.	0.011	0.020	0.011
Heated to 100°C.	0.035	0.026	0.016
Heated to 150°C.			0.037
Heated to 200°C.	0.124	0.064	0.060
Heated to 250°C.	0.189	0.079	0.064
Heated to 300°C.	0.059	0.053	0.036
Heated to 350°C.	0.052	0.055	0.048

TABLE 42

Lowering of the freezing point of different soils on heating to approximately 250°C.

SOIL	DEPRESSION OF FREEZING POINT; AVERAGE OF DUPLICATE DETERMINATIONS		NITROGEN AS NH_3 IN 100 GM. HEATED SOIL
	Not heated	Heated	
	°C.	°C.	mgm.
Peat	0.212	0.636	66.1
Muck	0.020	0.181	25.9
Waukesha silt loam	0.017	0.082	12.8
Virgin sandy loam	0.016	0.071	9.0
Fine sandy loam	0.018	0.053	10.2
Red clay	0.018	0.031	2.3
Norfolk sand	0.004	0.021	4.4

to various temperatures up to 250°C. followed by a decrease when heated to 300°C. A slight increase again on heating to 350°C. seems to indicate that at this high temperature marked decomposition of inorganic material has begun, following the destruction of the soluble organic matter. From the data presented in the last tables, there is shown to be a considerable degree of correlation between concentration of soil solution and the temperature of heating as far as any one soil is concerned, which condition is in turn correlated with such other factors as degree of toxicity, favorableness to fungus growth,

amount of ammonia produced, and color and odor of soil extracts. There seems to be no correlation, however, between concentration of soil extracts and these factors when different soils are compared. Determinations of the

TABLE 43

Temperatures developed in Dewar flasks with germinating wheat in extracts of heated soil

EXPERIMENT NUMBER	SOLUTIONS	TEMPERATURE REACHED						AVERAGE TEMPERATURE
		At start	After 1 day	After 2 days	After 3 days	After 4 days	After 5 days	
		Fine sandy loam extract						
		°C.	°C.	°C.	°C.	°C.	°C.	°C.
1	Redistilled H_2O	19.1	29.5	44.3	44.8	41.6	39.3	36.4
	Not heated	20.4	28.1	40.4	42.0	40.8	37.7	34.9
	Heated to 115°C.	21.4	31.3	42.5	42.6	41.3	38.3	36.2
	Heated to 250°C.	21.2	26.6	37.1	37.8	35.4	32.6	31.8
2	Redistilled H_2O	18.2	35.0	41.0	41.8	39.8	36.0	35.3
	Not heated	20.0	31.0	37.0	36.5	33.0	29.0	31.1
	Heated to 115°C.	20.7	33.7	39.2	41.7	39.2	38.0	35.6
	Heated to 250°C.	19.3	26.8	32.3	29.6	25.8	23.3	26.2
3	Redistilled H_2O	18.5	25.8	36.0	32.0	29.0		28.3
	Not heated	19.2	23.5	25.0	22.0	20.6		22.1
	Heated to 115°C.	19.2	25.0	28.1	24.0	22.0		23.7
	Heated to 250°C.	19.2	23.8	26.0	22.8	21.0		22.6
		Waukesha silt loam extract						
1	Redistilled H_2O	18.2	31.0	40.2	42.0	42.0	40.0	35.6
	Not heated	20.0	21.3	31.0	27.5	24.0	22.5	24.4
	Heated to 115°C.	20.1	29.0	34.0	31.0	27.0	24.5	27.6
	Heated to 250°C.	20.0	27.0	33.1	30.0	26.0	23.2	26.5
2	Redistilled H_2O	18.8	28.7	42.2	44.2	47.2	38.8	36.6
	Not heated	20.0	25.0	35.0	37.0	34.0	31.0	30.3
	Heated to 115°C.	20.2	26.5	38.5	40.0	40.5	36.5	33.7
	Heated to 250°C.	20.2	27.1	38.0	39.6	38.8	36.2	33.3
3	Redistilled H_2O	18.1	27.0	35.2	32.0	28.5		28.1
	Not heated	20.1	27.4	29.5	25.5	22.5		25.0
	Heated to 115°C.	19.0	26.8	33.5	30.1	26.7		27.3
	Heated to 250°C.	19.3	24.8	32.3	28.1	24.5		25.8

concentration of soil extracts were made from seven widely different soils heated to 250°C. (table 42). A wide variation in concentration will be noted, and although this appears to bear some relation to the amount of ammonia produced on heating, it is considerably less correlated with the toxicity of these extracts to seed germination.

In this connection it may be worth while mentioning briefly another method used in studying the behavior of soil extracts, with the hope of arriving at some conclusion as to the nature of their action upon seeds. This method was essentially that followed by Darsie, Elliott, and Peirce (10) in their study of the germination power of seeds in Dewar flasks by means of recording the temperature of respiration. A weighed quantity of wheat was placed in thermos bottles and sterilized with formaldehyde solution, after which equal amounts of extracts of unheated soil, soil heated to 115°C. and 250°C. was poured in the flasks. Redistilled water was used as checks. The thermometers were inserted through cotton plugs and into the wheat. The relatively small temperature differences secured are due largely to the fact that wheat, although cheap and convenient for this purpose, is relatively resistant to the action of heated soil extracts. If more susceptible seed had been used more marked results would no doubt have been secured. Some of the results are, however, presented in table 43 and serve to show the regularity of the results secured, in addition to corroborating results secured in actual seed germination tests. Comparing the average temperatures, it will be at once noted that the extract of an unheated soil is considerably less favorable to germination than pure water. Heating to 115°C., however, either destroys this toxicity in part or stimulates germination. Extracts of soil heated to 250°C. cause retardation of germination in wheat as measured by rise of temperature in thermos bottles and compared with the action of extracts of unheated soil.

Relation of toxic and beneficial properties.

It has been shown in this paper that a fairly marked correlation exists between seed germination and the toxic or beneficial action to plant growth when the same soil is heated to different temperatures, and that these are in turn correlated with the amount of ammonia present, as well as with the concentration of the soil solution. There is a strong presumption, therefore, that the property which causes simple retardation to plant growth is the same as the one which causes retarded seed germination. The property which causes "chemical" injury to plant growth, however, appears to be different from that causing retarded seed germination. Virgin sandy loam soil heated to 115°C. for 160 minutes, though extremely toxic to the growth of tomatoes reacted much the same as unheated soil in so far as lettuce or tomato seed germination is concerned. It is a difficult matter to prove definitely that the toxic agent causing retarded plant growth, and that retarding seed germination are the same, although the most reasonable presumption is that they are identical. There is, furthermore, seemingly no relation between the response of seeds to the toxic action and that of the early plant growth of any one plant. Lettuce which is for instance very susceptible to the toxicity of heated soils as far as seed germination is concerned, is relatively resistant

to the toxic agent during growth. Tomato plants, on the other hand, which are relatively susceptible during growth to the toxic properties, are more resistant in germination than lettuce seed. In general it may be said that no apparent striking correlation has been found to exist between seed germination and plant growth on soil heated to the same temperature. Traube (75) in working with acids and narcotics was also unable to find any constant relation between injury to germination and growth. In spite of this conclusion, however, one cannot fail to note exceptions. The seeds of the cereals are notably resistant to the agents toxic to germination, as are the cereal plants in their growth. Certain of the heated soils used, especially Waukesha silt loam, which is highly toxic to seed germination, is also relatively toxic to early plant growth, while others, especially fine sandy loam, not highly toxic to seed germination, are not very toxic to early plant growth.

TABLE 44

Production of ammonia in Waukesha silt loam soil heated to different temperatures on standing moist in greenhouse

SOIL TREATMENT	NITROGEN AS NH_3 IN 100 GM. DRY SOIL			TEST FOR NITRATES AFTER 18 DAYS
	Initial production	After 18 days "naturally" reinoculated	After 18 days artificially reinoculated	
	mgm.	*mgm.*	*mgm.*	
None		2.5	2.1	+
Heated to 50°C.		3.2	2.5	+ Strong
Heated to 100°C.	2.6	4.1	3.9	+ Strong
Heated to 150°C.	6.0	10.3	9.0	+
Heated to 200°C.	9.1	12.1	13.9	+
Heated to 250°C.	14.0	13.9	16.7	+ Weak
Heated to 300°C.	11.0	10.7	13.6	+ Weak
Heated to 350°C.	5.5	7.3	7.4	+ Weak

Regardless of the relation between the agents toxic to seed germination and plant growth, however, it may be well to consider the relation of the ammonia produced in the soil upon heating and subsequent to heating upon plant growth. All soils upon heating to different temperatures show usually an increasing toxicity to growth up to 250°C., which then falls rapidly and is practically nil at 350°C. While this is true for early growth, the final yields for most plants at least are usually greatest at 250°C., and fall off gradually at higher or lower temperatures of heating. With these facts only in mind, let us consider the ammonia relation in the soil under such conditions. Reference to table 44 shows a gradual increase in ammonia due to heating up to 250°C. but a falling off at higher temperatures. The period between the time of heating and the eighteenth day following may be taken as the average period during which toxicity to plant growth ordinarily occurs, since with many plants recovery from the toxic action may be well on the way after

3 weeks, especially at the lower temperatures of heating. Ammonia determinations were made after 18 days in both soils, in which inoculation with normal soil flora was made soon after cooling and where the soil was left to become "naturally" inoculated. These results show that after a period of 18 days, at least, soil heated to different temperatures and standing moist in the greenhouse increases rather than decreases in content of ammonia. These results were secured with an uncropped soil, and the question may arise as to what might be expected to happen on heated soils where crops are grown during this period of recovery. In an experiment Waukesha silt loam was heated to different temperatures ranging from 50°C. to 350°C. and planted to soybeans. It was found that the ammonia content was reduced to the greatest extent in the soils heated to the lowest temperatures (50°–150°C.) after 3 months. The soil heated to 200°C. and 250°C. retained the highest proportion of ammonia in relation to the amount produced by heating and these soils were still toxic to soybeans after 3 months and gave the smallest relative increase in yield. With radish, however, a more rapid reduction of ammonia content was produced, and the final yield was greatest on the soil heated to 250°C. The kind of crop grown apparently influences to a considerable extent the rate with which ammonia is removed from the soil, as well as the yield obtained from these soils. It is well known that heating the soil, at least to the higher temperatures, tends to reduce the nitrates present to very small amounts. Determinations made on Waukesha silt loam and muck by the colorimetric method showed that on heating to 250°C. the former was reduced from 2.75 mgm. nitrogen per 100 gm. soil to traces only, and in the latter cases from 2.28 mgm. to 1.19 mgm. Heating to 350°C. completely destroyed all nitrates. Qualitative determinations have shown that in highly heated soils increase in nitrates following heating is very slow, although it finally may become established. At any rate it is evident that the increased plant growth where it occurs relatively soon after heating soils to 250°–350°C., takes place in the presence of a greatly reduced supply of nitrate nitrogen but in an increased amount of ammoniacal nitrogen. Assuming ammonia to be present in sufficient amounts to be toxic to the early growth of plants, it is not difficult to conceive of the rapid recovery of plants resistant to its action owing to a decreasing amount (resulting from its use by the plant as well as to other changes) which finally reaches an optimum for the soil and for the plant, thus resulting in a very rapid increased growth followed by reduced rate of growth in the presence of a nitrogen supply below the optimum. For any one soil, the degree of toxicity, the time required for recovery, and the final increased yield are, then, largely dependent upon the temperature of heating and the susceptibility of the plants to the toxic (or beneficial) action.

That these soils respond to ammonia fertilization has been repeatedly noted and is illustrated in table 45 for tomatoes, where retardation was followed by marked increase in growth. The addition of ammonia as hydroxide to soils in different amounts, may, then, be regulated to such a point as to produce

an action quite similar to that resulting from heating the soils to various temperatures up to 250°C. Small amounts of ammonia show no retardation or stimulation, larger amounts very decided retardation. But the soils may finally recover completely from the injurious action and in the end show a markedly increased yield. Ammonium carbonate will produce similar results which in turn compare very well with the growth of tomatoes on soil heated to different temperatures (plate 7, fig. 2). Similar action may of course be obtained by other chemicals capable of being toxic, and later being volatilized or changed to a point where they may become a stimulant or a plant food. No clear case, however, of a response similar to the "chemical injury" of plants on heated soils has been obtained with pure chemicals.

That green plants are able to substitute ammoniacal nitrogen for nitrate nitrogen has been well established (26). It has also been shown that different plants vary in their ability to use ammoniacal nitrogen. Although the matter

TABLE 45

Yield of tomato vines on muck soil treated with different amounts of ammonia as ammonium carbonate

APPROXIMATE STRENGTH OF AMMONIA SOLUTION ADDED TO 2 KILOS SOIL	DRY WEIGHT	INCREASE	EARLY GROWTH
	gm.	*per cent*	
None	3.00		
0.1 per cent	8.75	191	Best; no retardation noted
0.2 per cent	13.30	243	Slight retardation
0.3 per cent	12.10	303	Marked retardation
0.4 per cent	11.25	275	First plants killed; reset; marked retardation

has not been investigated from this point of view, it seems reasonable to suppose that the differences in plants in their response to soil sterilization may be due in part to their capability of using ammoniacal nitrogen in the absence of nitrate nitrogen.

In this connection a study of the growth of plants on heated soils under aseptic conditions was made in order to determine the relation of the soil flora to the supply of plant food. Experiments in growing plants under sterile conditions in heated soil were started. Various forms of apparatus have been devised by investigators for growing plants under aseptic conditions, but these are ordinarily too complicated to permit the use of any considerable number of such structures. "Kellerman culture chambers" were finally selected for use in the experiments recorded here. These proved very satisfactory excepting for the fact that aeration is very poor under the conditions there afforded. It was found, therefore, that when a soil relatively toxic was heated or when plants relatively susceptible to toxic action were used, the time required for the plants to recover from the

injurious action was too long to permit a study of the beneficial action of the heated sterile soil on plant growth. Finally by selecting the least toxic soil (fine sandy loam), heating to 115°C. and planting to wheat, which is relatively resistant to the toxic action, it was found that fairly reliable results could be secured. That sterile conditions were obtained was demonstrated by transferring small bits of this soil with a sterile needle into bouillon tubes just before harvesting the crop. In the last experiment, the results of which are presented in table 46, one of the three flasks supposedly sterile had become infested with Penicillium but was apparently sterile insofar as bacteria were concerned. The increased growth on the reinoculated soil above that of the checks and sterile cultures is sufficient to be of considerable significance as indicating the relation of bacterial activity to the bene-

TABLE 46

Growth of wheat under sterile conditions as compared with unsterilized soil and sterilized and reinoculated soil

TREATMENT	NUMBER	DRY WEIGHT	AVERAGE	INCREASE
		gm.	*gm.*	*per cent*
No treatment	1	0.160		
	2	0.179		
	3	0.160	0.166	
Sterile soil	4	0.226		
	5	0.216	0.221	32.2
	6	*		
Sterilized and reinoculated	7	0.515		
	8	0.335		
	9	0.467	0.439	164.4

* This jar became accidentally infested with a fungus (Penicillium) and the yield was 0.482 gm.

ficial action of heated soils. The explanation of the results secured is not as simple as it may appear at first sight. Ordinarily it would be concluded that the increased growth on the reinoculated sterile soils is due almost entirely to increased bacterial activity in the sterilized soil as a result of new conditions which make this increased activity possible. The object of this experiment was to determine the relative importance of bacterial activity as compared with the direct chemical action of heat on soil in rendering food available for plants. It seems reasonable to assume that much of the increased plant growth on heated soils (especially of those heated to high temperatures) is due to direct chemical action in rendering plant food available. The results secured in growing plants under sterile conditions point quite strongly in this direction, since it was found that the plants grown in the sterile soil made an appreciable increase above the unheated checks in spite

of a toxic action sufficient to retard greatly the proper functioning of the root system. This condition was shown by the root systems in the sterile cultures which were very short, thick, small in total area, lacking in root hairs, penetrating only to shallow depths in the soil and generally unhealthy in appearance.

In the sterilized soils where reinoculation was made, the toxicity of the soil was apparently rapidly destroyed. The root system benefited thereby in quite a striking manner, being considerably better than that in the sterile checks or in the unheated soil (plate 8, fig. 2). Since all other factors were the same in the flasks except for the presence of soil organisms, it is evident that the destruction of the toxic substance was due in a large measure if not entirely, to their action. It seems extremely probable, therefore, that the increased growth in the reinoculated sterilized soil as compared with the sterile soil, was due in some considerable measure to the destruction of the toxic substance, which retarded the growth in sterile cultures but which was relatively rapidly destroyed or lost, or perhaps, even converted to plant food in the reinoculated soil cultures, and that the smaller growth in the sterile cultures was due as much to the toxic agent as to lack of increased food supply due to bacterial activity. This experiment illustrates further the important part played by soil organisms in benefiting sterilized soils due to the destruction of the toxic agent, making it possible for the plants to benefit sooner from the plant food rendered available as a result of the direct and indirect action of the heat upon the soil.

Conditions favoring development of microörganisms in heated soil

Turning to a consideration of the growth of fungi on heated soil, two possible hypotheses suggest themselves in the broadest sense. These are that a condition or substance unfavorable to fungus growth exists naturally in soil and is destroyed upon heating, or that new conditions or substances favorable to fungus growth are produced in heated soils. The former theory is essentially that of Kosaroff (35) who supposed that the soil contained certain toxins for fungi which are destroyed upon heating. Seaver and Clark (67, 68) have done a considerable amount of work in the support of the latter theory and conclude that the increased concentration of the soil solution favors fungus growth, which may at the same time be retarding in its influence upon the growth of green plants. Kosaroff's theory rests primarily upon the fact that the addition of the extract of a heated soil to an unheated soil will not permit of the growth of *Pyronema* upon the unheated soil. Seaver and Clark also obtained the same result. The writer was able, however, to obtain a good growth of *Pyronema* upon unheated soil by the addition of a heated soil extract following sterilization of the unheated soil by means of formaldehyde. It is highly improbable that a formalin drench (1–50) destroyed the soil toxin (and if it did it alone should permit of the growth of *Pyronema*),

but it did quite satisfactorily sterilize the soil for a time. It seems evident in view of the results previously described on the relation of the activity of soil microörganisms to the reduction of the toxic products produced in heated soil, that extracts of heated soils added to unheated soils are changed in character before the growth of *Pyronema* can establish itself. Although the formalin may have slightly increased the concentration of the soil solution (33) it is believed that its sterilizing properties were responsible for the changes produced which permitted the growth of *Pyronema* on heated soil extract added to unheated soil. This observation together with the findings of Seaver and Clark are believed sufficient to overthrow Kosaroff's arguments on the relation of soil toxins to the development of *Pyronema*.

That the concentration of food material as such plays an important part in the growth of fungi on heated soil as suggested by Seaver and Clark (67) seems doubtful in view of the following facts: the concentration of certain heated soils on which *Pyronema* grows readily may be considerably less than that of certain unheated soils or soils heated to 350°C. on which *Pyronema* will not establish itself. This is illustrated by the fact that *Pyronema* will make a good growth upon heated red clay or Norfolk sand or even relatively pure sand in which the concentration of the soil solution is relatively very low as compared with that of unheated peat or muck soil upon which *Pyronema* will not grow. Again the disappearance of the favorable condition for *Pyronema* from the soil seems to be considerably more rapid than the loss of concentration. At least, it is not to be expected that the food supply for the fungus is so rapidly diminished as to materially check the fungus growth after a period of only a few days. This statment needs of course, to be supported by the observation that more than one "crop" of fungus growth can occur upon any one heated soil.

That soils sterilized by heat at low temperatures or by antiseptics become a favorable medium for subsequent bacterial development has been shown repeatedly by various investigators beginning with the work of Hiltner and Störmer (25). The principal point of argument in recent years as to the effect of sterilization on the soil has been centered around the explanation of the cause of this increased bacterial activity. On account of the seemingly close correlation between the rate of bacterial development and of fungus growth on soils heated to different temperatures there apparently is no reason for distinguishing between the cause of the rapid development of these closely related microörganisms on sterilized soils. If this be true, then Russell's protozoan theory (64) would necessarily have to extend the activity of the phagocytic organisms to the limitation of fungus growth as well as to the limitation of bacterial development. The special favorableness which *Pyronema* finds for growth upon heated soils over that of other fungi is, however, apparently a matter for discussion aside from that of ordinary increased development of microörganisms in heated soils. The arguments of Seaver and Clark are apparently not concerned with this fact, but, using *Pyronema*

as an example, they attempt to explain why fungi in general find the soil a more favorable medium for growth. On the same assumption as previously made their conclusions might then be made to apply also to the explanation of increased bacterial activity in soil. The subject then finally resolves itself into two separate problems. (a) Why do microörganisms in general find a sterilized soil a more favorable medium for development? (b) Why does *Pyronema confluens* in particular find heated soils a more favorable medium for growth than do other fungi? The first problem will be dismissed with the simple statement that the writer's observations on a wide variety and type of heat sterilized soils have led to the opinion that in practically all cases the increased activity of microörganisms in sterilized soil can be explained on the basis of reduced competition (a wide variety of organisms are known to be negatively "chemotropic" to each other) in the presence of increased food supply without any special reference to the concentration of this food supply as such.

Seaver and Clark (68) assume as one basis for their theory on the growth of *Pyronema* in heated soil, that a concentration of soil solution favorable to fungi is unfavorable to green plants. The experiments of Raulin (57), and especially Bauman (3) who noted the relation of zinc salts to plants, and found that fungi are able to grow in solutions of zinc exceedingly toxic to higher plants, and Heald (23) who found the toxicity of carbolic and other acids to green plants greater than to fungi, illustrate the fact that certain fungi are relatively resistant to toxic substances. It is now generally recognized that the resistance of fungi to toxic agents is greater than that of green plants. It is not surprising, therefore, that fungi should grow on heated soils highly toxic to green plants regardless of the concentration of the soil solution, providing that these fungi find the substratum congenial because of reduced competition and increased food supply of either a general or a special nature. If we now reason a step further we should expect to discover that some fungi will find heated soils more favorable for growth than others, due to a modified food supply, as shown by Nikitinsky (45) and others, in the same way that certain green plants find heated soils more favorable for growth than others do since fungi as well as green plants differ in their food requirements. The fact remains, however that *Pyronema* is capable of reducing the toxicity to seed germination (and probably also to plant growth,) while growing on heated soil and at the same time of increasing the ammonia content of the soil. What relation the compound favorable to the growth of *Pyronema* has to the substance toxic to seed germination must, however, still remain obscure. The presumption is all in favor of it being a nitrogenous compound, but no good evidence exists on this point.

DISCUSSION OF RESULTS

Some of the conclusions drawn from the data presented in this paper have already been published in a preliminary paper (28). More data to support

the conclusions drawn have been secured since the publication of this first paper. On the other hand, some contradictory evidence on one point in particular has been obtained. That the instability of ammonium carbonates in heated soils, except when kept in a dry and unaerated condition, accounts for the gradual decrease of the injurious action is not supported by all the facts, since destruction of the carbonate was attributed to purely chemical or physical phenomena. The results secured since have shown the importance of microörganisms in the loss of toxicity. That the reduced toxicity is due to biological action upon ammonium carbonate, resulting in loss of gaseous ammonia, or to its fixation in the soil seems now most probable.

It seems evident from these studies that a large number of difficulties present themselves in the way of obtaining direct evidence upon the toxic action of the ammonia produced in heated soils. It has been felt in the course of the work that sufficient emphasis has not been placed upon quantitative determinations of the ammonia present, as such, for comparison with the toxicity of similar amounts in and out of the soil. On the other hand such evidence has seemed of minor value in view of the extraordinary influence of various chemical, biological, and physical processes in the soil on the ammonia relations. The extensive literature upon ammonia relations in soil abounds with evidence of the great variability of results secured in fertilization, ammonification, fixation, and absorption experiments. It seems worth while, however, to go to the literature to find confirmatory evidence for some of the conclusions presented in this paper.

With reference to toxicity of ammonia in soils to plant growth under conditions where it is reasonable to assume the amounts present are no greater than those produced in heated soils, mention may be made of the observations and experiments of Pitsch (54), Wagner (76), and Ehrenberg (14). Other investigators who have dealt with the susceptibility of seeds and plants to injury from ammonia in soils are Sigmund (69), Coupin (9), Ehrenberg (15), and Bokorny (4). Especially interesting in this connection are some of the results secured by Sigmund (69) and Ehrenberg (15) on the influence of zinc on soil. It has been shown that zinc is able to liberate ammonia from ammonium salts, which is then said to act corrosively on the plant roots through its hydroxyl ion, but on account of its easy dissociation, the ammonia partly volatilizes. Sterilization of the soil increases the injury while the action of the nitrate-forming organisms in removing ammonia compounds results in lessened or prevented injury. It appears, therefore, that we have in the action of zinc on soil a condition similar in many respects to that of heated soils.

As early as 1880, a very fundamental study was made by Nivet (46) on the relation of ammonia in the soil. This investigator found that ammonia occurs normally in soils as ammonium carbonate, and that the addition of ammonia in form of sulfate, results in ammonium carbonate being produced through its reaction with the calcium carbonate. The ammonium carbonate

being more or less unstable, considerable loss of ammonia from the soil may result, which loss varies greatly, however, with the nature of the soil. A great variation in the absorptive property of the soil was also noted in this connection, it being found that the ammonia given off from sandy soil was seven times as great as that from "humus" soil. In the presence of a considerable excess of pure carbon dioxide, the loss of ammonia by volatilization was reduced to an inappreciable amount. From the results of the investigations of Nivet (46) and others, it is evident that a consideration of the toxicity of ammonia in soils involves to a considerable extent its relations to the absorptive capacity present. The phenomena of absorption in soils has been known for nearly a century and many of the early workers as Way (77) had a good conception of the part played by this factor in heated soils, but more fundamental evidence from our standpoint has been brought together by recent workers, especially Richter (58), Wagner (76), Stoklasa (72), Prescott (56), and Cook (8). The most important facts advanced by these writers are as follows. Soils have a remarkable degree of power for absorbing gases, and especially ammonia, but they differ very widely in this capacity. In general the heavy types of soil, or those high in humus or vegetable matter, have a high absorptive capacity, and those of a light nature or low in vegetable matter have a low absorptive capacity. A striking variation from this rule, however, may be expected, and the actual or relative absorption of any soil can only be determined by actual trial. Absorption of ammonia is not, however, a simple physical process, but chemical and colloidal properties are also involved, together with many exterior influences, such as the temperature and moisture content of the soil. Chemical absorption may, for instance, result in the formation of an insoluble chemical compound. Colloidal matter increases the power of absorption. Calcium, especially calcium oxide, increases the absorptive power for ammonia in a decided way. Heating the soil decreases the absorption or fixation, a fact probably due largely to the action on humus and colloidal matter. High temperatures are generally unfavorable and low temperatures favorable to absorption, while increased moisture supply tends to permit of greater absorption.

With these facts in mind, it may be assumed that the following approximates the conditions in soil heated to 250°C. The maximum ammonia content is present in connection with a reduction in absorptive capacity of the soil. The amount of the measurable toxicity is then the result of the balance between the total ammonia produced on heating and the total absorptive capacity which exists in the heated soil. Heated soils with high ammonia content but also with high absorptive capacity, as is true in the case of peat, are therefore, less toxic than Waukesha silt loam with lower ammonia content, but with much lower absorptive capacity. Theoretically, a correlation should exist between the ammonia content and the absorptive capacity on the one hand and the toxicity to seed germination and plant growth on the other. The presence of numerous other disturbing factors renders such an

expectation practically unobtainable. The studies of Kanda (29) on the stimulatory action of metallic salts upon plant growth are merely cited to show the importance of such disturbing factors. In the case of copper sulfate, for instance, Kanda found it was difficult to get constant results in experiments because of the influence of such factors as the action of the salt upon the humus compounds of the soil, the influence of the time of the year, and especially temperature and moisture changes in the atmosphere. It was found that in a cold, moist atmosphere, the toxic action was much less than in a warm, dry atmosphere. Other meteorological and climatological factors were also found to influence results. In a similar manner, the influence of environmental factors, some of which are as yet not clearly understood, appear to complicate the data secured on seed germination and plant growth on heated soils.

The action of ammonia or ammonium compounds as plant food has been demonstrated (26). Lyon and Bizzell (42), Kelley and McGeorge (30) and others, as well as results of the author have also shown that nitrates are reduced in heated soils and that they are practically non-existent on soil heated to 250°C. Furthermore, according to some writers, heated soils become unfavorable for bacterial activity and although this conclusion is doubtful in all cases, it is at least probable that it takes some time before nitrification can reestablish itself to a decided extent. In the absence of nitrates, but with large supplies of ammonia present, it is probable that ammonia is being used directly by the plant as a source of nitrogen where plant growth occurs. That cereals in the presence of ammonia at least could develop in soils free from nitrates was concluded by Pitsch (54) as early as 1887. The researches of Kossowitsch (36) and Hutchinson and Miller (26) have added sufficient evidence to make it appear unquestionable that agricultural plants of various kinds can produce normal growth when supplied with nitrogen as ammonia in the absence of nitrates.

There is apparently room for difference of opinion as to the form of ammonia existing in heated soils. Ammonia as such, probably is never present in normal soils. When ammonium salts are added to soils, it is generally believed that they pass through several stages of decomposition and nitrification before being taken up by the plants. It is known that under ordinary conditions much ammonia exists in the soil as ammonium carbonate. On the other hand, it also appears that some of the ammonia enters into very stable combinations, from which it is no more readily separable (61). Eliminating for the present the biological factor in the soil, such as may be expected to occur in soils while heating or in highly heated soils stored dry, one is confronted with a chemical or at most a physico-chemical problem. There appears to be several reasons for the assumption that much of the ammonia produced in heated soils exists as some form of carbonate of ammonia. These are briefly: (a) The high production of carbon dixoide coincident with that of ammonia production may be expected to result in the combination of the two when cool-

ing to lower temperatures. (b) The stability of the high ammonia content in the soil when kept dry in the presence of reduced absorptive capacity argues against its existence as a gas. (c) The comparative ease with which ammonia is driven off from a soil extract at low temperatures indicates that it exists as an unstable compound. (d) The slight increase of acidity in heated soils indicates an excess of an acid radical, most probably carbon dioxide, which is produced in large amounts on heating, and hence reduces the possibility of ammonia remaining in the soil for any length of time as a free base.

When heated soils are kept moist and aerated, the carbonate of ammonia present is no doubt gradually dissociated into ammonia and carbon dioxide, only to be reabsorbed by the soil, changed or fixed into other forms by biological or other action, or possibly lost in small amounts to the atmosphere, depending upon the favorableness of the soil for the activities concerned. These changes in heated soils together with the ammonia removed by growing plants, where present, explain the loss of ammonia from heated soils and hence may account for reduced toxicity. As a matter of fact, however, an increase in ammonia actually occurs for a period of time following sterilization of the soil in spite of gradually reduced toxicity. Here the assumption must be made that the ammonia exists at this critical stage, not as toxic gas, but in various delicate stages of transition between humus compounds and ammonia on the one hand and between ammonia and nitrates on the other, and that these compounds are reduced to ammonia in the determinations of total ammonia present in the soil. In this process of transition, microörganisms play the main rôle, and we have shown that microörganisms are largely responsible for the destruction of the toxic property in the soil. It is of course equally probable that certain organic compounds, which may or may not be transition products of ammonia, and which result from decomposition of humus on heating, may be the toxic property involved. In contradiction of this we have only the evidence of the "qualitative" similarity of the action of ammonia and its compounds on seed germination as compared with that of highly toxic heated soils or the extracts of such heated soils. This evidence, together with the fact that ammonia or ammonium salts added to soils in correct amounts may produce marked similarity in action to that produced by heated soil on seed germination and plant growth, constitutes the main evidence for the conclusion that ammonia plays a large rôle in both the toxic and beneficial action of heated soils to seed germination and plant growth, although it may not be the only factor concerned. That all seeds and plants do not respond in a similar manner to heated soils is believed to be due to the difference in the selective permeability of the seed coat or of the plasma membrane to the toxic agents. This may also explain the ability of certain plants to assimilate nitrogen in the form of ammonia or ammonium salts either in the absence of, or in a reduced supply of other forms of nitrogen and hence may account in part for the beneficial action of heated soils on certain plants while they appear at the same time to be injurious to other species of plants.

SUMMARY

1. The purpose of this investigation has been primarily to arrive at some conclusions as to the nature of the action of sterilized soils upon plant growth, in order that their use in soil biological and phytopathological research as well as in prophylactic measures may be more clearly understood and more productive of reliable results.

2. Practically all soils when sterilized in the ordinary manner by heat at temperatures approximating 100-115°C. produce temporary retardation to seed germination and plant growth followed by increased rate of growth. The extent of this action varies very greatly with the soil, seed, and plants used and with the environmental conditions existing. To study the factors concerned, 7 different soils were subjected to widely varying treatments and conditions, and their action on various seeds and plants determined. Although some of the results secured are of a corroboratory nature, these have been extended or limited in their application.

3. On heating a soil to different temperatures, it was usually found that a gradual increase in toxicity occurs to seed germination and to early plant growth which reaches its maximum at approximately 250°C., but gradually decreases to practically no toxicity on soils heated to 350°C. or above.

4. The time required for recovery from this toxic action is usually directly proportional to the intensity of toxicity produced, but the final beneficial action is often greatest on the soils exhibiting the greatest injurious action on early plant growth.

5. Different soils vary markedly in their behavior upon heating to the same temperatures both in toxicity and in beneficial action to seeds and plants, and this is not seemingly correlated with any single distinguishing character in the soil, but is correlated rather with the balance of all the factors concerned.

6. Seeds vary greatly in their sensitiveness to the toxic action. Lettuce and clover seed are for instance, very susceptible to the toxic substance, whereas seeds of wheat, buckwheat or flax are very resistant. The degree of sensitiveness of seeds is roughly characteristic of their genetic relationship. The Gramineae and the Cucubitaceae are usually resistant and the Leguminoseae and Solanaceae are, as far as determined, more susceptible.

7. With the seeds resistant to the toxic action marked acceleration of rate of germination may occur on even highly heated soils. This is no doubt another expression of the same substances that cause retardation in more susceptible seeds. Seeds classed as susceptible may on the other hand show accelerated germination on soils not productive of high toxicity on heating or upon heating soils to low temperatures only.

8. Growing plants differ markedly in their sensitiveness to the action of heated soils. Soils very toxic to certain plants, such as tomatoes, may be very beneficial to others such as wheat. The similarity of the behavior of growing

plants and germinating seeds in this respect suggests that the injurious and beneficial substance in both cases may be the same. Toxicity to seed germination, however, is seemingly not always correlated with toxicity to plant growth and vice versa. Furthermore, sensitiveness of seeds to the toxic agent in germination is not indicative of the behavior of the same species in its growth on the same soil.

9. The growth of fungi on heated soil is correlated with the toxicity to seed germination and plant growth on any one soil. The growth of *Pyronema* especially has been studied. This and other fungi and apparently bacteria also grow best in soil heated to 250°C., diminishing in rate and profuseness of growth at lower or higher temperatures of heating.

10. The ammonia content of a soil heated to different temperatures is highest on heating to about 250°C. and diminishes gradually at higher and lower temperatures of heating. This is also true for the concentration of the soil solution. Ammonia content and concentration of the soil solution are therefore roughly correlated with the degree of toxicity to seed germination and early plant growth and the extent of the beneficial action to late growth of green plants and to growth of lower microörganisms in any one soil.

11. There is apparently, however, no correlation between these factors when different soils are compared to each other. The toxicity of heated Waukesha silt loam with a relatively low ammonia content is, for instance, much greater than that of heated peat with a relatively much higher ammonia content. The absorptive capacity of the two soils is, however, vastly different, and this in turn markedly influences the action of toxic compounds produced in the soil. In water extracts of heated soils, the toxicity to seed germination is more directly proportional to the ammonia content. It seems that the toxicity of the soil is, therefore, not only determined by the amount of the toxic agent produced by heating, but also by the absorptive capacity of the soil for the toxic agent as well as by a number of other factors, the additive and subtractive value of which gives a balance of toxicity very difficult to properly analyze.

12. The addition of ammonia as such to soil in varying amounts may be made to produce a condition similar in many respects to that produced by heated soils on seed germination and plant growth.

13. Very similar "qualitative" reactions can be produced with certain seeds on highly toxic heated soils or their extracts and with certain strengths of ammonia. These reactions do not seem to be reproducible with chemicals other than ammonia or ammonium salts.

14. It is believed that much of the toxic action in heated soils is due to the ammonia produced and that it exists in the heated dry soils largely as ammonium carbonate, which is, however, gradually decomposed under normal growing conditions.

15. All the toxic properties in heated soils are not, however, believed to be the same. Certain changes termed "chemical" injuries are seemingly due to quite different causes than ordinary retardation.

16. The toxic property is volatile and is destroyed or changed to non-toxic compounds in soils kept under normal growing conditions. This has been shown to be due to the activity of ordinary soil flora, which, however, may apparently at the same time increase the amount of ammonia present. *Pyronema confluens* has also been shown to be efficient in both these respects. The reduced toxicity in the presence of increased ammonia content in the soil is believed to be explained by its existence in various delicate transition stages rather than as true ammonia due to the activity of soil organisms and that these transition products are broken down when the ammonia determinations are made. The reduction of the toxic property to seed germination and plant growth by the activity of soil flora has been shown and is contrary to Pickering's conclusion that loss of toxicity in storage of heated soils is an oxidation process which may go on under aseptic conditions.

17. The beneficial action of highly heated soils is believed to be due in a considerable measure to the ammonia liberated on heating, since increased growth may result in almost total absence of nitrates or in heated soil under aseptic conditions in spite of a considerable toxic action upon the roots. The fact that certain green plants are able to take up their nitrogen in the form of ammonia is believed partially to explain the variation of sensitiveness of plants to heated soils.

18. The temperature of the soil is an important factor in determining the extent of the toxic and beneficial action to plant growth. The toxic action disappears more slowly and is more destructive at low soil temperatures (below about 25°C.) than at higher temperatures.

19. Observations on the growth of *Pyronema* seem to indicate that the favorableness of heated soils to its growth is not entirely one of concentration of soil solution, as argued by Seaver and Clark. *Pyronema* will grow on heated soils very low in concentration as compared with other unheated soils on which growth never occurs. All microörganisms appear to grow better on the soils of higher concentration of soil solution, owing to increased food supply, but the type of organism and the extent of its growth will vary with the competition at hand and the kind of food materials present. Fungi differ in their food requirements in much the same way as green plants, and heating the soil no doubt produces chemical substances especially favorable to the growth of *Pyronema*.

20. The conclusions drawn here are considered to apply particularly to highly heated soils, although it is not believed that any fundamental difference exists between ordinary steam sterilized soils and highly heated soils reinoculated with normal soil flora.

21. Although the injurious action of heated soils on plant growth has been brought into the foreground in this paper, it is not desired to leave the impression that heat sterilized soils are of questionable value in research and practice. The opposite is rather true and little hesitancy need be felt in recommending steam sterilization of soil for practical purposes or for use in research problems

where it is necessary to eliminate certain organisms from the soil. But, one must be prepared to expect a short period of retardation of growth followed by a beneficial action, and in special cases, with certain soils or plants or under certain environmental conditions, a very marked interference with the normal development of plants.

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PLATE 1

The Influence of Heated Soils on Seed Germination and Plant Growth

Fig. 1. Showing the difference in response of different soils on heating to 110°C. on the growth of tomatoes. *A*, Check, soil not heated; *B*, heated soil. Pots 1A–1B, Miami silt loam; pots 2A–2B, muck; pots 3A–3B, red clay; pots 4A–4B, greenhouse compost; pots 5A–5B, peat; pots 6A–6B, virgin sandy loam.

Fig. 2. Effect of heating virgin sandy loam to different temperatures, showing the retarding influence on plant growth. *1*, Not heated; *2*, heated to 50°C.; *3*, 100°C.; *4*, 150°C.; *5*, 200°C.; *6*, 250°C.; *7*, 300°C.; *8*, 350°C.

Fig. 3. Growth of radish on muck soil heated to different temperatures illustrating the beneficial action to late plant growth. *A*, Not heated; *B*, heated to 115°C.; *C*, heated to 250°C.; *D*, heated to 350°C.

FIG. 1

FIG. 2

FIG. 3

PLATE 2

The Influence of Heated Soils on Seed Germination and Plant Growth

Fig. 1. Growth of soy beans and tobacco on Waukesha silt loam soil heated to different temperatures, showing the beneficial action of the lower and higher temperatures of heating on soybeans, but the retarded recovery at 150°–250°, whereas the tobacco has recovered and shows beneficial action at these temperatures. *A*, Not heated; *B*, heated to 50°C.; *C*, 100°C.; *D*, 150°C.; *E*, 200°C.; *F*, 250°C.; *G*, 300°C.; *H*, 350°C.

Fig. 2. Showing the variation in behavior of different crops on virgin sandy loam soil heated to 115°C. *A*, Tomatoes on unheated soil; *B*, tomatoes on heated soil; *C*, wheat on unheated soil; *D*, wheat on heated soil; *E*, buckwheat on unheated soil; *F*, buckwheat on heated soil.

Fig. 1

Fig. 2

PLATE 3

The Influence of Heated Soils on Seed Germination and Plant Growth

Fig. 1. The growth of tomatoes on muck soil heated for different lengths of time at 115°C., showing an appreciable beneficial action at the longer times of heating. *1*, Not heated; *2* heated 10 minutes; *3*, 20 minutes; *4*, 40 minutes; *5*, 80 minutes; *6*, 160 minutes.

Fig. 2. The growth of tomatoes on virgin sandy loam heated for different lengths of time at 115°C. showing a decided injurious action at the longer times of heating. *A*, Not heated; *B*, heated 10 minutes; *C*, 20 minutes; *D*, 40 minutes; *E*, 80 minutes; *F*, 160 minutes.

Fig. 1

Fig. 2

PLATE 4

The Influence of Heated Soils on Seed Germination and Plant Growth

Fig. 1. Showing the influence of soil temperature on the extent of the toxic action of heated Miami silt loam soil on the growth of tobacco. *1A*, Not heated and grown at 28–29°C.; *1B*, heated to 110°C. and grown at 28–29°C.; *2A*, not heated and grown at 23–24°C.; *2B*, heated to 110°C. and grown at 23–24°C.

Fig. 2. Showing the influence of soil temperature on the extent of the toxic action of virgin sandy loam on the growth of tomatoes. *1A*, Not sterilized soil and grown at 17–18°C.; *1B*, sterilized soil and grown at 17–18°C.; *2A*, not sterilized soil and grown at 23–24°C.; *2B*, sterilized soil and grown at 23–24°C.; *3A*, not sterilized soil and grown at 28–29°C.; *3B*, sterilized soil and grown at 28–29°C.

Fig. 1

Fig. 2

PLATE 5

The Influence of Heated Soils on Seed Germination and Plant Growth

Fig. 1. Illustrating the influence of soil temperature on the toxic action of heated soil on the roots of tomato plants. The higher soil temperatures greatly reduced the extent of the toxic action.

Fig. 2. "Chemical" injury of heated greenhouse compost (manure and sod mixture) on tomato plant. This injury occurred suddenly after plant had been growing normally for some time. *A*, Check, soil not heated; *B*, soil heated to 110°C.

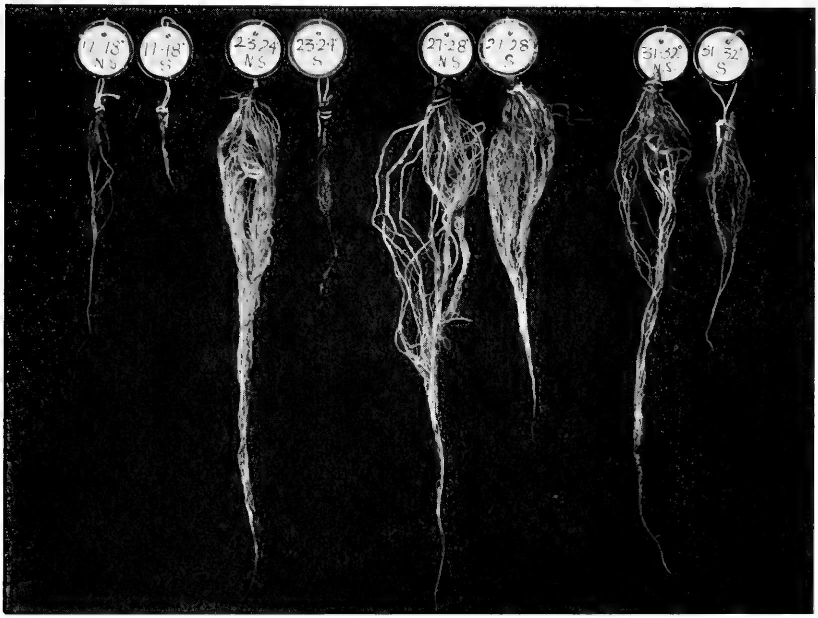

Fig. 1

Fig. 2

PLATE 6

The Influence of Heated Soils on Seed Germination and Plant Growth

"Chemical" injuries to the foliage of various plants as a result of growing on heat sterilized soils. *A*, Mottled leaf of tomatoes quite common on certain heated soils; *B*, collapse of leaflets and petiole of tomato grown on heated soil; *C*, "leaf spots" of tobacco; *D*, marginal spotting on soybeans; *E*, injury to midribs and veins of leaflets of soybeans resulting in curling; *F*, "specking" of cowpeas.

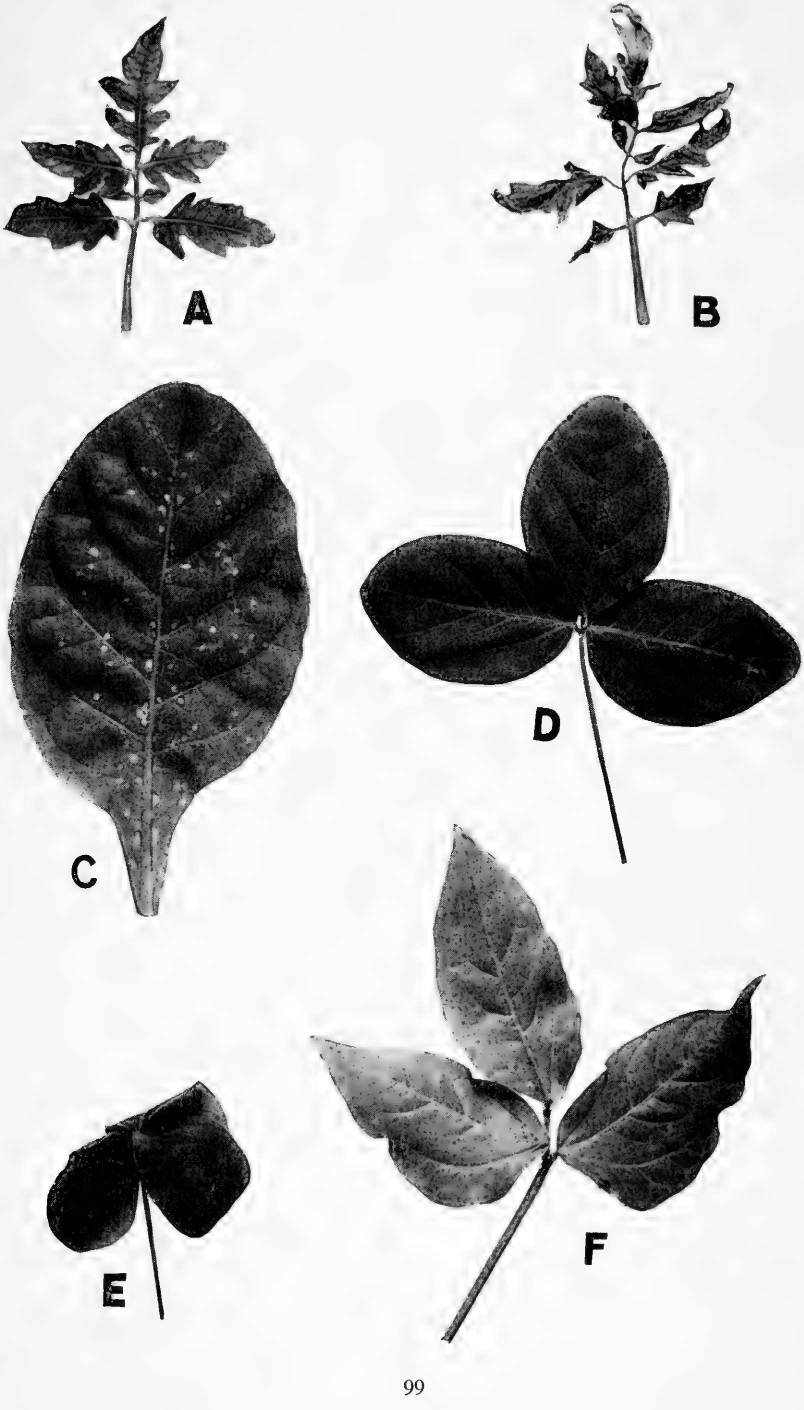
A
B
C
D
E
F

PLATE 7

The Influence of Heated Soils on Seed Germination and Plant Growth

Fig. 1. The growth of *Pyronema* on soil heated to different temperatures. *A*, Not heated; *B*, heated to 50°C.; *C*, 100°C.; *D*, 150°C.; *E*, 200°C.; *F*, 250°C.; *G*, 300°C.; *H*, 350°C.

Fig. 2. Growth of tomatoes on silt loam soil heated to different temperatures, as compared with treatment with varying amounts of ammonia, as ammonium carbonate, illustrating the similarity in responses obtainable. *1A*, Not heated; *2A*, heated to 150°C.; *3A*, heated to 200°C.; *4A*, heated to 250°C.; *1B*, no treatment; *2B*, treated with 0.1 per cent ammonium carbonate; *3B*, treated with 0.2 per cent ammonium carbonate; *4B*, treated with 0.4 per cent ammonium carbonate.

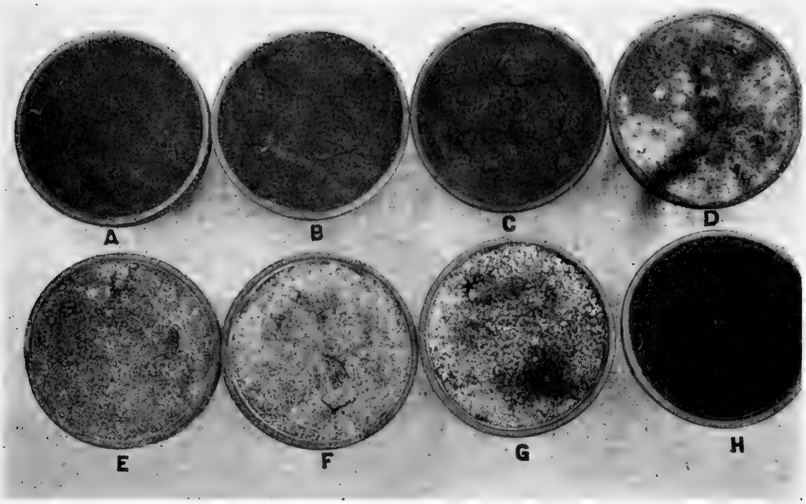

Fig. 1

Fig. 2

PLATE 8

The Influence of Heated Soils on Seed Germination and Plant Growth

Fig. 1. Growth of wheat on fine sandy loam under various conditions with respect to activity of microörganisms. *A*, Sterilized soil reïnoculated with normal soil flora; *B*, setrilized soil under aseptic conditions; *C*, unsterilized soil check.

Fig. 2. Showing the relative development of tops and roots of wheat grown on heated and unheated soil under varying conditions with respect to activity of microörganisms. *A*, Sterilized soil reinoculated with normal soil flora; *B*, sterilized soil, and aseptic conditions; *C*, unsterilized soil check. Note especially the stunted root systems in *B*, but better aerial growth than in *C* in spite of the toxic action. The loss of the toxic action on roots in reinoculated soil is shown by comparing *A* with *B*.

Fig. 1

Fig 2